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MEDICAL SECTION OF NATIONAL TUBERCULOSIS ASSOCIATION

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THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

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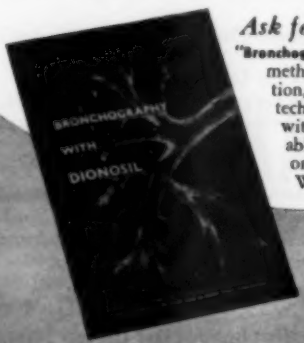

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2. Harper, A. E.: Amino Acid Imbalance, Toxicities and Antagonisms, Nutrition Rev. *14*:225 (Aug.) 1956.
3. Amino Acid Requirements of Adult Man, Nutrition Rev. *14*:232 (Aug.) 1956.
4. Amino Acid Imbalance and Supplementation, Editorial, J.A.M.A. *161*:884 (June 30) 1956. Council on Foods and Nutrition, American Medical Association: Importance of Amino Acid Balance in Nutrition, J.A.M.A. *158*:655 (June 25) 1955.

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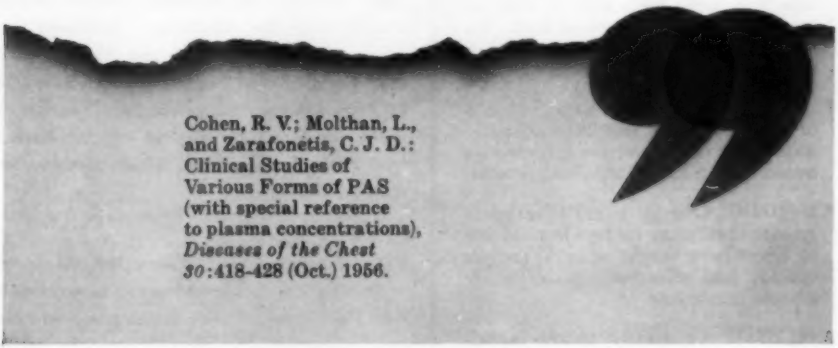

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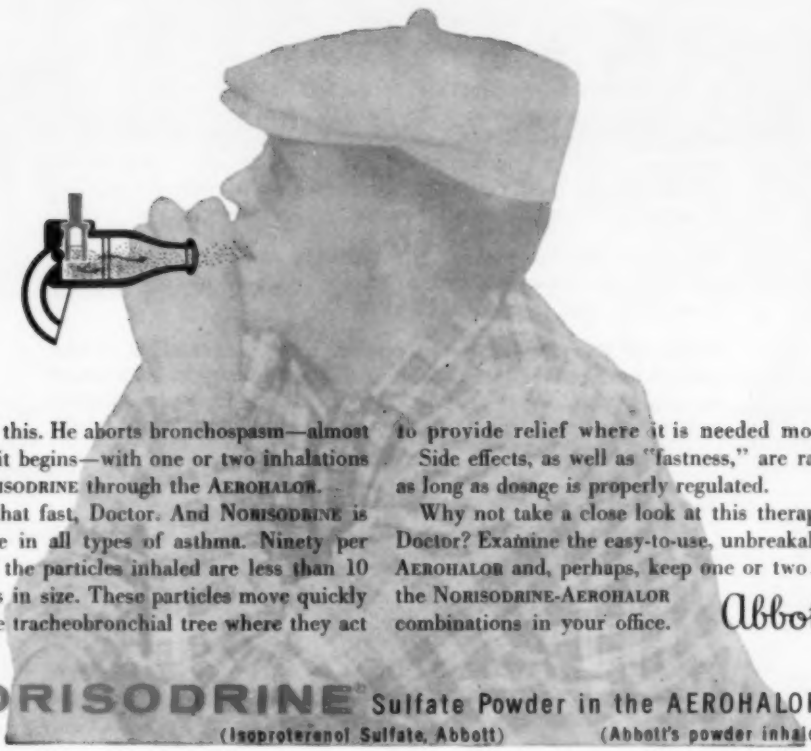

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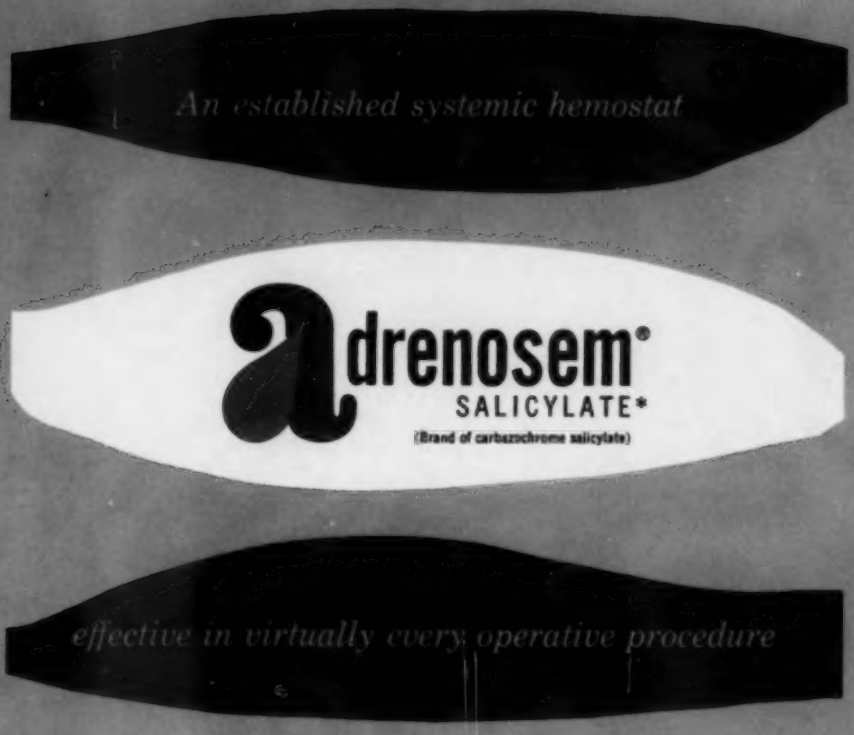

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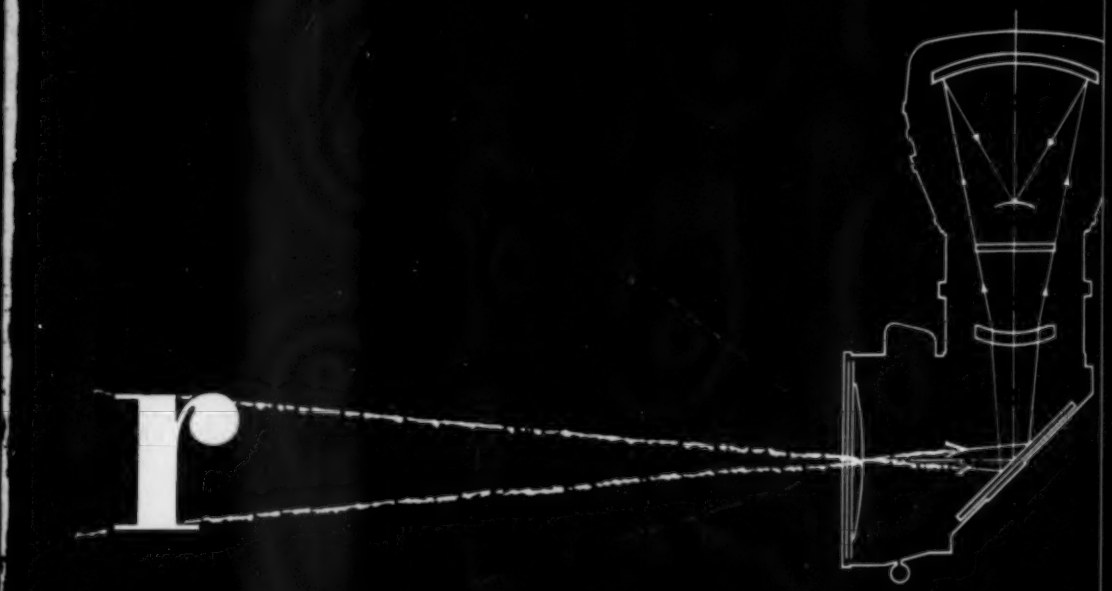

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THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

VOLUME 76 *August, 1957* NUMBER 2

ON TAPING THE CHEST[1]

ROBERT W. CARTON AND ENDEL SEPP

(Received for publication January 28, 1957)

INTRODUCTION

A variety of therapeutic procedures alter ventilation and lung movement by restricting movement of the chest wall. These include thoracoplasty (1) and various chest-strapping procedures. The latter have been used clinically for years (2) without any exact knowledge of the effect produced on the underlying lung. Although the dangers of pneumonia and atelectasis due to inadequate ventilation have been postulated as a sequel to strapping the chest (3), this relationship has been no better understood than that between strapping one hemithorax and the relief of underlying pleural pain.

The present report consists of an analysis of the connection between taping the chest and the ventilation of the lungs and an application of this information to clinical problems.

METHODS

Vital capacity and maximal breathing capacity were examined, using a 9 liter Collins spirometer, and two of these instruments were used to record ventilation through the Carlens bronchospirometry catheter. The position of the catheter was checked by fluoroscopy, and in most instances roentgenograms were taken to ensure proper position. All gas volumes were corrected to body temperature and saturation at ambient pressure.

When strapping was required, adhesive strips three inches in width were applied over three-quarters of the chest in deep expiration, as described by Donaldson (3) and as used generally in clinical medicine. This layer ran from the midclavicular line anteriorly across the chest and around to a corresponding line posteriorly.

To measure relative diaphragmatic excursion, posterior-anterior roentgenograms of the chest were taken in forced inspiration and in forced expiration with identical technique. The roentgenograms were superimposed so that the spinous processes of the upper thoracic vertebrae matched, and then relative diaphragmatic displacement was measured along a vertical line on either side parallel to the spinal column and 7.5 cm. from it. The distortions mentioned by Wade (4), (*1*) vertical displacement of the diaphragmatic shadow because of straightening of the spine on forced inspiration; (*2*) geometric distortions because of the varying angle between beam source, diaphragm, and plate, were disregarded because they were approximately the same for the two sides.

[1] From the Presbyterian-St. Luke's Hospital, the Chicago State Tuberculosis Sanitarium, and the University of Illinois College of Medicine, Chicago, Illinois.

Tape was applied to patients with pleurisy in the same manner as to the other subjects. Analysis of the degree of pleural pain present was made by questioning. After a control period, tape was first applied to the side opposite the pain and left in place for two hours. The patient was then asked about his symptoms, the tape was removed, and then the process was repeated on the side of the pain. No medication or other treatment was given during the test periods.

Results

The Relation of Restriction of the Chest Wall to Distribution of Ventilation

Eight patients suffering from moderately advanced and far advanced pulmonary tuberculosis were examined prior to surgical treatment. Measurements of ventilation of the individual lungs were made before and after taping one side of the chest; in this way each patient served as his own control. The results are given in table 1.

Seven of the 8 patients showed reduction in the sum of the vital capacities after taping, but in only 5 of 7 was the percentage drop greater on the taped side. Generally the reduction in vital capacity was rather similar between the two lungs.

When minute volume ventilation was measured, 6 of the 8 patients showed a greater percentage drop on the taped side, but again the changes were similar between the two sides.

The Relation of Restriction of the Chest Wall to Over-all Ventilation

The ventilation of 9 healthy male medical students was measured before and after strapping one hemithorax. In this way the effects of debility were minimized and full cooperation was assured. It may be seen in table 2 that this type of

TABLE 1

Differential Ventilation Before and After Strapping One Hemithorax
Patients with Tuberculosis

	Side Strapped	Vital Capacity—Control		Vital Capacity After Strapping as a Percentage of Control Value		Minute Volume—Control in Liters Per Minute		Minute Volume After Strapping, as a Percentage of Control Value		Change in Ventilation in Favor of Unstrapped Side	
		Right	Left	Right	Left	Right	Left	Right	Left	VC*	Minute Volume
		cc.	*cc.*	*per cent*	*per cent*	*liters*	*liters*	*per cent*	*per cent*		
WL	R	1,050	1,900	92.4	96.3	3.2	4.3	106	111	Yes	Yes
ML	R	1,140	790	79.0	83.5	5.5	3.0	105	100	Yes	No
BF	R	1,740	1,340	89.7	88.2	6.8	2.9	99	100	No	Yes
ER	R	1,640	2,045	96.0	100.0	5.8	7.6	90	84	Yes	No
RS	L	860	1,780	94.7	92.7	3.7	6.9	95	86	Yes	Yes
LM	L	850	1,520	100.0	96.7	3.4	6.2	100	86	Yes	Yes
OS	L	380	1,880	76.6	82.0	0.7	3.9	128	108	No	Yes
EG	L	1,660	1,500	103.7	105.3	8.4	7.5	100	100	No	—

* VC = Vital capacity.

TABLE 2

OVER-ALL VENTILATION AFTER STRAPPING ONE HEMITHORAX
Medical Students

Subject	Side Strapped	Vital Capacity* After Strapping	Maximal Breathing Capacity* After Strapping
		per cent	*per cent*
M C	Right	94	90
J F	Right	95	98
O B	Left	95	89
R E	Right	86	83
J S	Left	95	112
N H	Left	87	77
A S	Right	98	71
D S	Left	84	78
J S	Left	95	103
Mean		92	89

* As a percentage of the control value before strapping.

restriction reduces vital capacity an average of 8 per cent and maximal breathing capacity, 11 per cent. The students volunteered the information that the binding felt tight and inspiration became uncomfortable long before they were forced to stop this maneuver.

The Relation Between Restriction of the Chest Wall and Movement of the Diaphragm

In view of the slight effect on ventilation of strapping one hemithorax, one wonders whether movement of the diaphragm might not compensate for alterations in movement of the chest wall, as suggested by Bray (5). To test this idea, chest roentgenograms of 5 normal males were made in full inspiration and expiration before and after strapping. The results are given in table 3. In all instances the changes were slight, generally 1 cm. or less, and no side-specific pattern pointed to augmentation or inhibition of diaphragmatic motion by chest strapping.

TABLE 3

EFFECT OF TAPING ON MOTION OF THE DIAPHRAGM
Normal Subjects

Patient	Control Excursion of Dome of Diaphragm		Side Taped	After Strapping Excursion of Dome of Diaphragm		Difference Control: Test Readiing in Cm.	
	Right	Left		Right	Left	Right	Left
	cm.	*cm.*		*cm.*	*cm.*		
ES	8.6	7.7	Left	7.6	7.4	1.0	0.3
RWC	6.4	5.4	Right	5.6	5.4	0.8	0.0
JS	6.3	7.1	Left	6.6	6.4	−0.3	+0.7
DM	6.7	7.4	Right	5.7	5.7	1.0	1.7
GC	7.2	6.9	Left	7.3	6.9	−0.1	0.0

TABLE 4

RELIEF OF PLEURAL PAIN BY TAPING THE CHEST
Men with Fibrinous Pleurisy

Patient	Location of Pain: Side	Degree of Pain (4 + being the most)		
		Control	Hemithorax Opposite Pain Taped	Same Hemithorax as Pain Taped
RA	Left	4+	2+	4+
RC	Left	4+	1+	4+
WM	Right	4+	3+	1+
TW	Left	2+	1+	0
MH	Right	4+	2+	1+
LD	Right	3+	1+	1+
VR	Left	3+	2+	1+

Chest Strapping in the Relief of "Pleural" Pain

Seven patients suffering from pneumonia and pleurisy were seen at the Cook County Hospital.[2] The patients were diagnosed as having fibrinous pleurisy by a typical onset, pain, and friction rub. In each case roentgenographic evidence of pneumonia was present, and in most of them pneumococci were recovered from the sputum or blood. All were men between fifteen and seventy years of age.

The results (table 4) show that in 2 of the 7 cases strapping applied to the side *opposite* the pain was more effective than that applied to the side of the pain. In all cases some amelioration of pain was obtained by strapping the side opposite the pain. The results also show a general lessening of the pain with taping, in accordance with clinical experience.

DISCUSSION

From the data presented a picture can be drawn of the patient's ability to ventilate his lungs after his chest is taped. The ability to draw a deep breath is limited first by a sensation of tightness and then, to a slight extent, by the actual physical limitation of the adhesive bandage. As this deep breath is being drawn, the two underlying lungs are expanding and the two leaves of the diaphragm fall almost equally. This sequence is in accord with that recorded by Pinner, Leiner, and Zavod (6) in their study of 3 cases.

One is entitled to ask why ventilation is not reduced more effectively by chest strapping? Two answers seem apparent: (*1*) Motion of the diaphragm is not much affected. (*2*) The loose tissues over the thoracic cage "give" even after the first sensation of tightness is appreciated. From the data above, there does not seem to be much reason to worry about specific retention of secretions in a taped hemithorax, with consequent pneumonia or atelectasis. Adequate breath can be drawn for coughing.

Motion of the visceral pleura across the parietal pleura is dependent upon descent of the diaphragm and also upon movement of the thoracic cage. In the

[2] These patients were seen through the courtesy of Dr. Eugene Traut.

subjects movement of the diaphragm did not show any consistent pattern of change. Volume filling of the lungs was only slightly affected, making it seem likely that motion of the pleural surfaces across each other was affected only at the extremes of its range.

Why should taping of three-fourths of the circumference of the chest be effective in the relief of pleural pain? With this procedure there is little difference in motion between the two lungs, as measured either by ventilation studies or by vertical motion of the diaphragm. Is this a "side-specific" effect, or does it depend upon restriction of movement of the chest as a whole, or upon other causes? It has been shown repeatedly (5, 7–9) that the rubbing together of inflamed pleural surfaces is not a complete explanation of "pleural pain" in pleurisy. The present data lead to the belief that the thorax continues to move as a whole in spite of tape applied to part of it, and that this tape limits inspiration more by warning against a deep breath than by actual physical limitation.

Summary

After strapping one hemithorax, distribution of ventilation showed a change, giving a slightly increased proportion to the untaped side. Over-all vital capacity was reduced 8 per cent and maximal breathing capacity, 11 per cent. Strapping one hemithorax did not produce any consistent pattern of change in motion of the diaphragm.

Strapping applied to the chest for "pleural pain" is not uniformly specific as to side, but appears to act by inhibiting the extremes of motion of the chest as a whole. There appears no basis for the belief that tape applied to one hemithorax in expiration will specifically encourage pneumonia and atelectasis in the underlying lung.

Sumario

Sobre la Tórax

Después de fajar un hemitórax, la distribución de la ventilación reveló una alteración que daba una proporción ligeramente mayor al lado no puncionado. La capacidad vital en conjunto se redujo en 8 por ciento y la capacidad respiratoria máxima en 11 por ciento. El fajamiento del hemitórax no introdujo ningún patrón constante de alteración en los movimientos del diafragma.

El fajamiento aplicado al tórax para el "dolor pleural" no es uniformemente específico en cuanto al lado, pero parece obrar inhibiendo los extremos de movimiento del tórax en conjunto. No parece que tenga fundamento la idea de que el vendaje aplicado a un hemitórax en la espiración alentará específicamente la neumonía y la atelectasia en el pulmón subyacente.

Resume

A propos de l'immobilisation thoracique par bandage contentif

A la suite de l'application d'un bandage contentif sur un hémithorax, la répartition de la ventilation était modifiée dans le sens d'une légère augmentation du pourcentage du côté non comprimé. La capacité vitale totale était réduite de 8% et la capacité de ventilation maxima de 11%. Le bandage appliqué sur un hémithorax ne modifiait pas de façon constante les modalités des mouvements du diaphragme.

Le bandage contentif du thorax contre "l'algie pleurale" est dépourvu d'action vraiment spécifique sur un côté particulier, il semble agir sur les mouvements du thorax dans leur ensemble. La croyance qu'un bandage contentif appliqué sur un hémithorax en expiration puisse électivement favoriser la pneumonie ou l'atélectasie du poumon sous-jacent paraît sans fondement.

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ROENTGENOGRAPHIC PATTERNS IN HISTOPLASMOSIS[1, 2]

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INTRODUCTION

Histoplasmosis, once considered to be a rare generalized disease which was invariably fatal, is now known to be an asymptomatic disorder (1–3) in many instances. It is a systemic disease caused by the fungus *Histoplasma capsulatum*, usually manifested by granulomatous lesions in lymph nodes, spleen, liver, lungs, and bone marrow. In symptomatic cases the clinical findings include fever, cough, weight loss, ulcerations of the oral mucosa and gastrointestinal tract, hepatosplenomegaly, anemia, and leukopenia (4, 5). Although the first cases were reported in Panama and the disease has been reported from twenty-four countries, the bulk of the reports in the literature stem from cases observed in the endemic region of the United States, i. e., the Mississippi and Ohio River basins. Within this area, up to 90 per cent of the population gives a positive reaction to the histoplasmin skin test (6–8).

Numerous reports have appeared clarifying the epidemiologic, clinical, and pathologic phases of the disease (1, 2, 5, 9), but the roentgenographic aspects have not received the attention they deserve. The variety of roentgenographic manifestations reported in this disease has prompted the present review of the roentgenograms in a group of 28 cases in an attempt to classify the roentgenographic findings.

The 28 cases in the present study were selected from several hundred in the files of various hospitals in the Cincinnati area, on the basis of the degree of certainty of the diagnosis. It is believed that, if the requirement of cultural proof in each case of histoplasmosis is rigidly maintained, many subclinical forms will be ignored and our concept of the disease will be a distorted one.

CRITERIA FOR DIAGNOSIS

Certain criteria were established for the inclusion of cases in the present series. Cases of intrathoracic lesions, clinical manifestations, and course compatible with histoplasmosis, but in which the only supporting evidence were positive histoplasmin and negative tuberculin skin tests, were classified as *suggestive* and, with the exception of 2 cases of disseminated miliary calcifications, were excluded from the series. Cases of conversion of the histoplasmin skin test and/or a positive complement-fixation test with a high or rising titer were considered *presumptive* and were included in the series. Cases in which a positive culture was obtained

[1] From the Department of Radiology, University of Cincinnati College of Medicine, Cincinnati General Hospital, and Jewish Hospital, Cincinnati, Ohio.

[2] This study was supported in part by a research grant (E-576) from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service.

from the blood stream, sputum, et cetera, and those in which *H. capsulatum* was demonstrated microscopically in the tissue were considered *proved* and were also included in the series.

It should be noted that, in Cincinnati, 15 per cent of children have positive histoplasmin skin tests at the age of five, and 80 per cent have positive tests by the time they are eighteen. However, not all patients with *active* histoplasmosis show positive skin tests. In particular, the acute disseminated form is commonly associated with a negative test. While the critical titer of the complement-fixation test, i. e., the level at which the diagnosis of histoplasmosis is certain, has not yet been established, high titers and especially rising titers are considered reliable evidence of active infection with this organism. Low titers may persist after a previous histoplasmic infection, so that the serologic diagnosis of histoplasmosis is not as clear cut as might be desired, and the diagnostic value of the test cannot be compared with that of the demonstration of the organism.

ROENTGENOGRAPHIC CLASSIFICATION

The following roentgenographic classification of histoplasmosis has been devised:

I. Arrested or healing (with calcification)
  A. Primary complex
  B. Disseminated
  C. Splenic
  D. Solitary intrathoracic
II. Benign active
  A. Peripheral pulmonary infiltrate with lymphadenopathy
  B. Disseminated lesions and segmental involvement (with mild clinical symptoms)
  C. Intrathoracic lymphadenopathy alone
III. Acute disseminated histoplasmosis
  A. Miliary (or small nodular) type
  B. Miliary with superimposed localized infiltrate (with severe clinical symptoms)
  C. Hepatosplenomegaly
IV. Chronic progressive histoplasmosis
  A. Fibrocavitary
  B. Round or "coin" lesions

ILLUSTRATIVE CASE REPORTS

Arrested or Healing Histoplasmosis

Primary complex:

Case 1. An essentially asymptomatic 11-year-old girl with physical findings of patent ductus arteriosus reacted positively to histoplasmin and negatively to tuberculin.[2] As shown in the chest roentgenogram (figure 1), the primary complex consisted of a rounded peripheral calcification measuring 0.5 cm. in diameter in the left upper lobe and a large (3 cm.) aggregate of calcium in the left paratracheal lymph node region. A partially calcified node was re-

[2] Second-strength PPD, 0.0001.

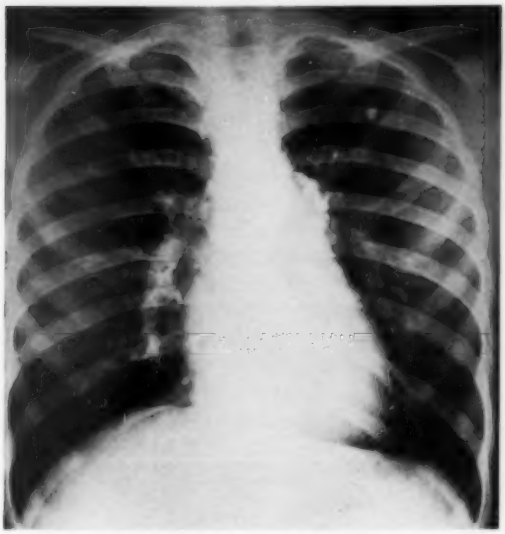


FIG. 1, *Case 1*. Chest roentgenogram of an 11-year-old girl. A calcified left hilar lymph node was removed at operation for uncomplimented patency of the ductus arteriosus; many nonviable fungi, *H. capsulatum*, were found in this node. The primary complex consisted of a rounded peripheral calcification in the left upper lung and a large aggregate of calcified left paratracheal lymph nodes (Am. J. Med. 1955, *19*, 410).

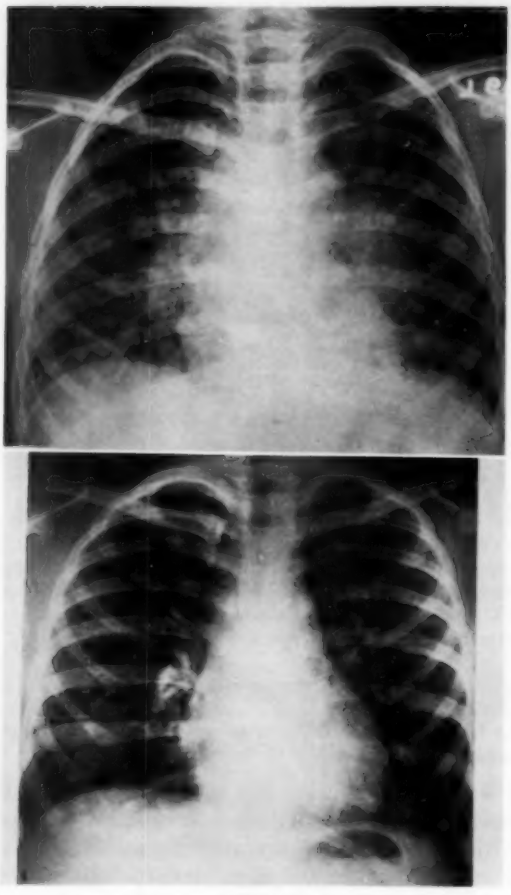
moved during operation for the patent ductus and revealed many nonviable fungi characteristic of *H. capsulatum*. In another child (*Case 2*), however, a large (2.5 cm.) irregular calcified left lower lobe focus which followed an atelectatic "pneumonia" was associated with only barely perceptible calcifications in the left hilus.

Disseminated miliary calcifications: The development of pulmonary calcifications was observed in 3 children with miliary and multiple nodular lesions (*Cases 3*, *4*, and *16*). In 2 instances calcification appeared approximately three years—and in one case four years—after the first chest roentgenogram was obtained. These children were six, eight, and ten years of age, respectively. Two had symptoms of severe upper respiratory infection at onset. The third child was hospitalized because of acute rheumatic fever, and the disseminated pulmonary lesions were an incidental finding. All 3 patients had positive histoplasmin skin tests and were negative reactors to tuberculin.[4] In one case there was a reversal of the histoplasmin skin test from negative to positive five months after the onset of the acute clinical symptoms; in the other 2 there was a positive complement-fixation test (figures 2 and 3).

Splenic and solitary intrathoracic lesions:

Case 5. A 71-year-old woman presented no pulmonary symptoms but had long-standing arteriosclerotic heart disease. Roentgenograms revealed a large round density with a central

[4] First-strength PPD, 0.00001 and second-strength PPD, 0.0001.



Case 16

FIG. 2 (Upper). Chest roentgenogram of an 8-year-old girl who on admission, January 3, 1949, showed disseminated rather ill-defined patches of density in both lungs. Note the marked bilateral hilar adenopathy. Histoplasmin skin sensitivity was present; the PPD was negative. The complement-fixation test for *H. capsulatum* was positive, 1:320.

FIG. 3 (Lower). Follow-up roentgenogram of January 26, 1952. Definite calcification of the disseminated lesions was identified three years after the acute illness. Note calcification in the right hilar lymph node.

calcification and a peripheral halo in the left upper lung. There were also innumerable round calcified densities in the splenic region. The patient subsequently died and was examined at autopsy. The partially calcified lesion (1.2 cm. in diameter) was found in the left upper lobe of the lung. In this lesion and also in the calcified splenic foci, numerous yeast forms characteristic of *H. capsulatum* were demonstrated.

Comment: Multiple splenic calcific densities are now considered to be almost diagnostic of past infection with histoplasmosis; this is true only if there are more than five discrete nodules or, when less than five, if they have a concentric structure (10).

Benign Active Histoplasmosis

Peripheral infiltrate and hilar adenopathy: Three patients (*Cases 6, 8,* and *9*) ranged in age from twenty to twenty-five years; their illness began with chills and fever. The liver was palpable two fingerbreadths below the costal margin in one case, but the spleen was not enlarged in any case. Symptoms had been present from twenty-eight hours to several days. The chest roentgenogram showed a single density in the peripheral lung field in 2 cases, while two foci were seen in the third. Bilateral hilar adenopathy was observed in 2 cases and ipsilateral hilar lymph node enlargement in the third. The pulmonary infiltrates were round to oval and measured from 0.5 to 2.0 cm. in diameter.

In one of these cases, a student nurse who had recurrent chills and fever over a period of seven months, a normal chest roentgenogram was obtained initially. Seven months after the onset of illness a poorly defined density in the right midlung field was noted. Successive roentgenograms showed no change in the paren-

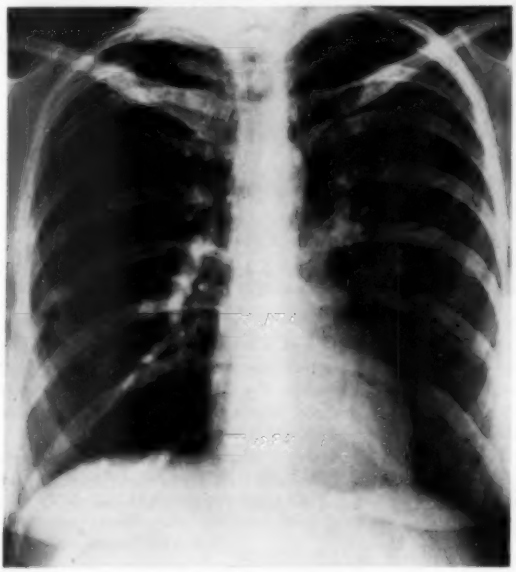
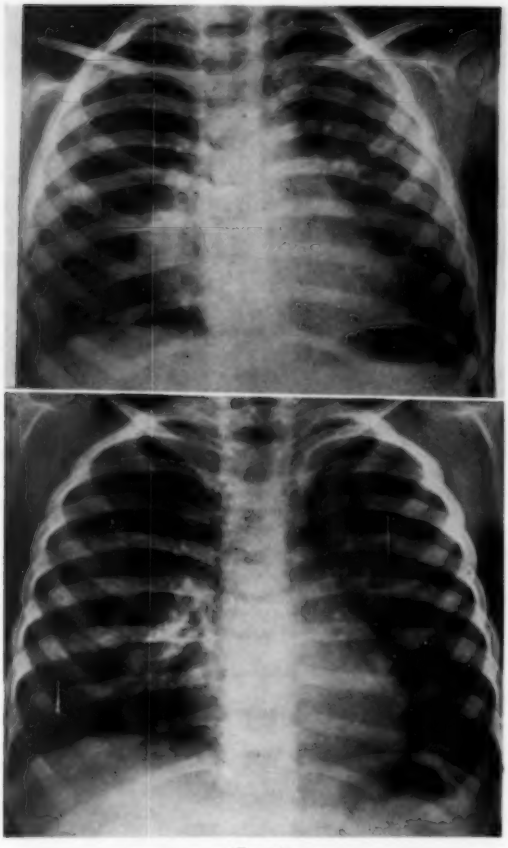


FIG. 4, *Case 6*. Chest roentgenogram of a 20-year-old student nurse. Seven months after the initial symptoms, a poorly defined oval-shaped infiltrate made its appearance in the right midlung field. Successive roentgenograms showed little change in the lesion until nine months after its appearance, when beginning calcification was noted. Biopsy of lymph nodes was performed; *H. capsulatum* was cultured from these.



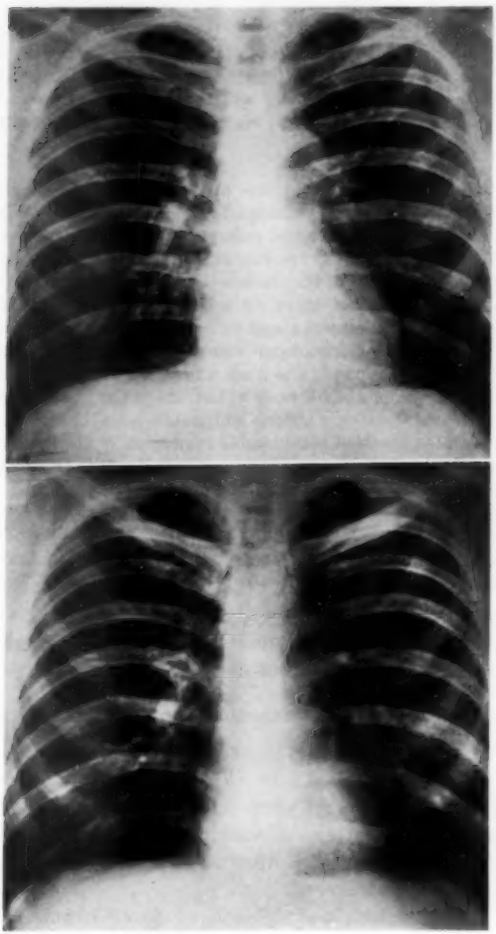
Case 7

FIG. 5 (Upper). Chest roentgenogram of a female child, 3 years and 6 months old, obtained in October, 1949. Note the dense right paracardiac infiltration with generalized small nodular and miliary densities in both lungs, the latter not well demonstrated in the photograph. Right hilar adenopathy of marked degree was present. The right heart border was obscured, indicating right middle lobe involvement.

FIG. 6 (Lower). Roentgenogram obtained on January 10, 1951. The right hilum had regressed in size, appearing less dense. There was a return of sharp contour to the hilar border. Numerous nodular densities persisted in both lungs. Cultures from bone marrow were positive for *H. capsulatum*.

chymal lesion until calcification appeared nine months later (figure 4). In the 2 other instances the infiltrate was completely resorbed after four months and two years, respectively, with gradual concurrent diminution of the hilar adenopathy.

Disseminated lesions and segmental infiltrate: Case 7 (figures 5 and 6) represents an example of dense infiltrate that involved the right middle lobe of a child. In addition, there were bilateral miliary and small nodular (4–5 mm.) parenchymal



Case 10

FIG. 7 (Upper). Chest roentgenogram, obtained June 16, 1955, of a 28-year-old white male, former resident of Massachusetts who had moved to Cincinnati one year previously. This film showed marked right and left hilar adenopathy with no evidence of a pulmonary infiltrate. The histoplasmin skin test was positive; the PPD, negative. A complement-fixation test for *H. capsulatum* was positive in dilution 1:16. Scalene node biopsy revealed noncaseating granuloma (question of sarcoid arose).

FIG. 8 (Lower). Roentgenogram of January 30, 1956. The hilar lymph nodes were markedly decreased in size.

densities diffusely distributed in both lungs, and right hilar lymphadenopathy. This case illustrates a segmental type of involvement similar to that of bacterial pneumonia. Of special interest is the combination of segmental consolidation and disseminated lesions.

Intrathoracic lymphadenopathy alone:

Case 10. A 28-year-old male who had moved to Cincinnati from Massachusetts twelve months previously was admitted with a febrile upper respiratory illness. A roentgenogram showed marked bilateral hilar adenopathy with no evidence of parenchymal infiltrate (figure 7). Six months later almost complete regression of the hilar nodes was noted (figure 8).

Case 11. Bilateral hilar lymphadenopathy was observed in a 6-year-old child. No parenchymal infiltrate was seen. The child was admitted to the hospital on September 30, 1954, with a history of low-grade fever and rapid enlargement of a firm shiny 4 cm. mass in the suprasternal notch, lying between the trachea and manubrium. An admission roentgenogram showed marked displacement of the trachea to the left and posteriorly, with compression by a retrosternal mediastinal mass extending down to the supracardiac region. Esophageal displacement to the left was noted on barium swallow. Because of the clinically obvious tracheal obstruction, a tracheotomy was performed. An inflammatory mass containing 10 ml. of thick pus was found. The mass was excised and the postoperative course was favorable. On recurrence of a small mass at the site of the tracheotomy, drainage was again performed and revealed a sterile abscess which subsequently healed. Roentgenograms obtained two months later revealed considerable regression of the mediastinal mass. Two rounded right mediastinal masses remained, however. In August, 1955, the upper mass had almost disappeared while the lower one remained unchanged.

In all 6 cases of benign active histoplasmosis, a positive histoplasmin skin test was obtained, while the PPD reaction was negative.[5] Gastric washings and sputum examinations were repeatedly negative for tubercle bacilli. In 3 patients *H. capsulatum* was demonstrated by culture (table 1); all 3 patients also presented positive complement-fixation tests. In the other 3 cases, positive complement-fixation tests was obtained but no cultural proof.

Acute Disseminated Histoplasmosis

The clinical data and roentgenograms of 8 cases of acute disseminated histoplasmosis were reviewed (*Cases 12* to *19*). Seven infections were in children ranging from five months to eight years of age; the other was in a 17-year-old girl. Five ran a short clinical course varying from eleven to thirty days and terminated fatally; one patient died after eight months of illness (*Case 19*). An 8-year-old child (*Case 16*) recovered.

The clinical manifestations were prolonged fever (from 99.5° to 106°F.), anorexia and, in 2 cases, diarrhea. A severe degree of anemia, in some cases as low as 5.3 to 7.7 gm. of hemoglobin, was found. Massive hepatosplenomegaly was present in all but one case, the child who recovered; her liver was only slightly enlarged and the spleen not at all. Leukopenia was observed in 4 instances.

[5] PPD tuberculin was employed exclusively:

Case 6—first-strength, 0.0002
second-strength, 0.05
Case 7—first-strength, 0.00001
second-strength, 0.0001
Case 8—first-strength, 0.0002
second-strength, 0.05
Case 9—first-strength, 0.0002
second-strength, 0.0025
Case 10—first-strength, 0.0002
second-strength, 0.05
Case 11—first-strength, 0.0002
second-strength, 0.0025

TABLE 1

BENIGN ACTIVE HISTOPLASMOSIS

Case	Age, Color, Sex	Complement-Fixation Test	Positive Culture	Duration of Residence in Endemic Area	Roentgenographic Findings	Remarks
Proved						
6	20 years, W. F.	Positive	From lymph nodes	Since birth	Rounded discrete infiltrate, right midlung field; right hilar adenopathy	
7	3½ years, W. F.	Positive 1:20	From bone marrow ×3	Since birth	Right paracardiac infiltrate and small nodular and miliary densities bilaterally; right hilar adenomegaly	
8	23 years, W. M.	"Strongly positive"	From peripheral blood	14 months	Two nodular infiltrates RUL and RLL; bilateral adenomegaly	
Presumptive						
9	25 years, W. F.	Positive	No	7 months	Circumscribed left midlung infiltrate; marked left hilar node enlargement	
10	28 years, W. M.	Positive 1:16	No	12 months	Bilateral hilar node enlargement	Scalene node: noncaseating granuloma. No fungi or acid-fast bacilli demonstrated.
11	6 years, N. M.	Positive 1:32	No	Since birth	Massive (upper) mediastinal lymphadenomegaly	Suprasternal abscess: nonspecific granuloma. No caseation or giant cells. No acid-fast bacilli or fungus on cultures.

FIG. 9, *Case 14*. Chest roentgenogram of a 2-year-old girl showed disseminated densities in the right and numerous densities in the left upper and midlung fields. Right and left hilar adenopathy was prominent. An extensive right perihilar infiltrate was present. The right heart border was obscured. An infiltrate with visible bronchial tree was seen behind the left heart shadow. A right interlobar exudate was also observed. A bone marrow examination revealed innumerable yeast cells of *H. capsulatum*. The patient died after a very short illness.

The histoplasmin skin test, performed in 5 cases, was positive in only 2 of them. The diagnosis was confirmed by culture of *H. capsulatum* in 6 cases and by direct smear of bone marrow in one. No organisms were demonstrated in the child who recovered. However, her histoplasmin skin test was repeatedly positive and the PPD, 0.0001 mg., was negative. Complement-fixation tests were also repeatedly positive (1:320). Three years later disseminated pulmonary clacifications appeared.

Miliary (or small nodular) type: Small nodules varying in diameter from 1 to 5 mm. were present in 6 of the 8 patients. The nodules varied considerably in size and were distributed rather evenly throughout both lungs.

Miliary with superimposed localized infiltrate: In one child (*Case 14*), confluent densities were seen in the right middle and left lower lobes (figure 9). In this patient and in one other (*Case 12*) a slight thickening of the right minor fissure suggested pleural exudate. In another case (*Case 13*), in addition to a dense miliary involvement of both lungs, a large, localized, rounded density was seen in the left lower lobe. At autopsy this proved to be a histoplasmic granuloma. Associated fibrocaseous and calcareous tracheal and bronchial lymph nodes were observed.

In another child (*Case 15*), whose two siblings were reported to have previously died of "leukemia," a history of hepatosplenomegaly, intermittent low-grade fever, and pallor was obtained. Roentgenograms showed a large aggregate of right hilar lymph nodes, markedly enlarged paratracheal nodes, and miliary densities diffusely distributed in both lungs. Moderate hilar adenopathy was

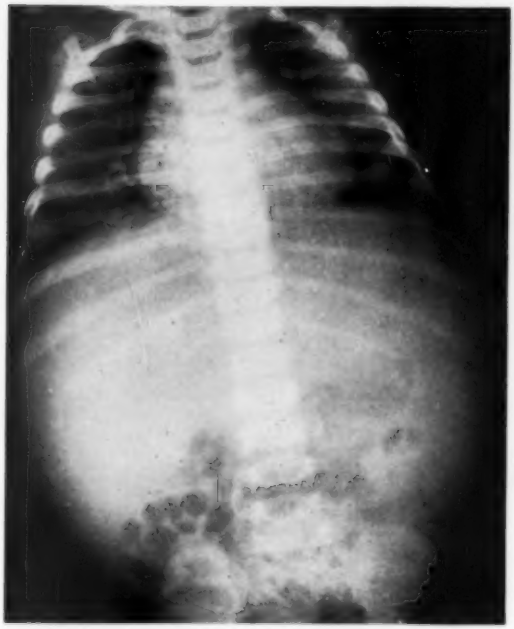


FIG. 10, *Case 18*. Roentgenogram of a 5-month-old male infant with acute systemic histoplasmosis, demonstrating massive hepatosplenomegaly. The liver and splenic margins were each on physical examination found to be three to four fingerbreadths below the costal margins. The lung fields were clear. The infant died one month after admission.

observed in all cases of disseminated lesions; however, the above case represented an exceptionally massive example.

The fatal case of a 17-year-old girl (*Case 17*) merits special interest in that the clinical illness was dominated by an exfoliative dermatitis. Eleven months later disseminated miliary densities involving the lower two-thirds of both lungs appeared. Moderate right hilar adenopathy was also present. Although the roentgenographic examination suggested no pleural effusion, on thoracentesis a moderate amount of fluid was recovered. *H. capsulatum* was cultured from this exudate and from the blood stream. Necropsy confirmed the presence of histoplasmosis in the lung. The presence of an old caseous focus in the apex of the right lower lobe suggested that the disease originated in the lung.

Hepatosplenomegaly: Two patients (*Cases 18 and 19*), a 5-month-old infant[6] and a 4-year-old child, respectively, demonstrated well that in the acute systemic form of the disease the lungs may be spared. Hepatosplenomegaly may be very striking in these cases, both clinically and roentgenographically. Autopsies confirmed the absence of pulmonary lesions and involvement of the liver and spleen in both cases (figure 10).

[6] *Case 18* is presented through the kindness of Dr. George Renner, Jr.

FIG. 11, *Case 22*. Chest roentgenogram of a 76-year-old asymptomatic man whose routine roentgenogram showed numerous large thin-walled left upper lobe cavities resembling large emphysematous bullae; a few were seen in the right apex also. Note marked thickening of the left upper lobe pleura. Numerous roentgenograms obtained during the following twenty-four months showed no remarkable change in the size of the cavities; however, later, with the onset of clinical manifestations, fluid levels were noted as well as patchy densities in the left lower and right upper and midlung fields. Note displacement of the trachea to the left and elevation of the left hilum. Numerous positive yeast cells of *H. capsulatum* were obtained.

Chronic Progressive Histoplasmosis

Fibrocavitary: The clinical and roentgenographic findings in 6 cases of progressive histoplasmosis (*Cases 20* to *25*) are here presented. The patients' ages ranged from fifty-six to seventy-nine years. It is to be noted that this age distribution is considerably higher than in other forms of the disease, a finding that has been noted by others (12). There were five males and one female in the group.

The clinical course was that of a chronic debilitating respiratory disease, and varied from several months to four years in duration. The predominant clinical findings in the group were fever, chills, dyspnea, cough productive of copious amounts of sputum, and progressive wasting. One patient (*Case 22*) was asymptomatic (figure 11).

In all 6 cases, sputum and/or bronchial washings were positive on culture for *H. capsulatum*. In 3 patients the complement-fixation tests were positive for Histoplasma antigen in titers of 1:160, 1:256, and 1:2,048. These 3 patients had upper lobe fibrocavitary disease. In 2 cases the complement-fixation tests were

FIG. 12, *Case 23*. Chest roentgenogram, May 26, 1954, of a 61-year-old white male boiler tender who, after onset of clinical symptoms of increasingly progressive dyspnea and productive cough in 1950, was hospitalized after an acute respiratory episode. Sputum smears and cultures were negative for acid-fast bacilli but positive for *H. capsulatum*. A complement-fixation test was positive, 1:2,048. Note extensive bilateral infiltrates in the upper lung fields with cavitation in the left upper lobe and bullous emphysema particularly in the right upper lobe. Both hila were elevated. Four days later massive right pneumothorax developed. Collapse involved the right lower and middle lobes primarily. He died five months later.

negative, while in one case the test was not performed. The histoplasmin tests of 4 patients in whom skin tests were performed, were positive in 3 and negative in one while the tuberculin tests were positive in 2 and negative in 2.[7] One patient (*Case 24*), with a positive histoplasmin skin test and positive tuberculin, was a 76-year-old male, who earlier in his disease presented several buccal mucosal lesions. On biopsy of these mucosal lesions, *H. capsulatum* was isolated. Although the chest roentgenograms initially were not remarkable, later a supervening tuberculous infection occurred, and an infiltrate appeared in the left upper lobe of the lung. Acid-fast bacilli were demonstrated on direct sputum examination and culture. Autopsy revealed only pulmonary tuberculosis and no evidence of *H. capsulatum* in the lungs.

[7] *Case 20*—PPD, 0.0025
Case 23—PPD, 0.0025; both negative

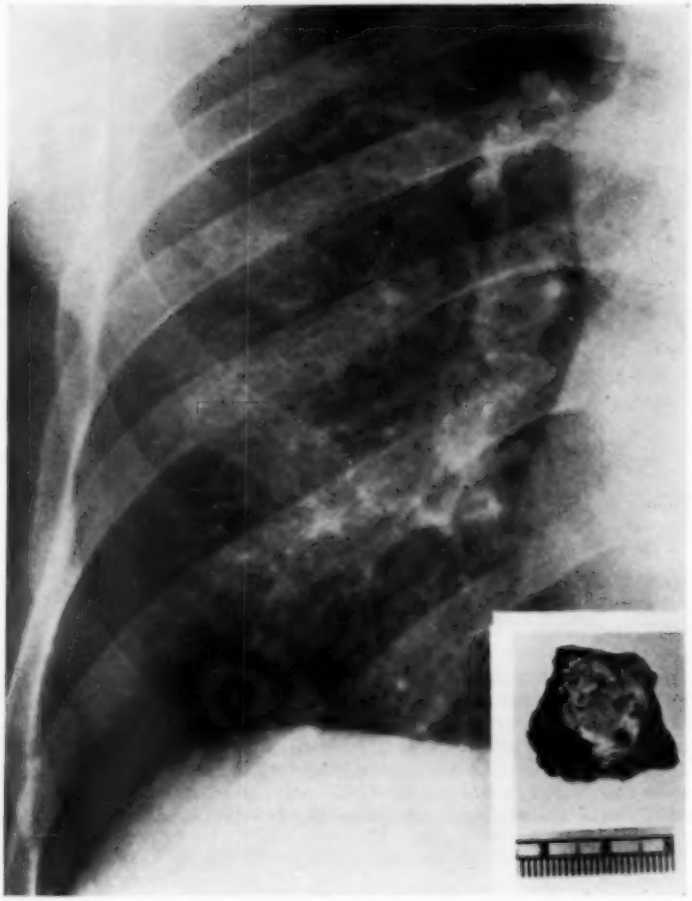


FIG. 13, *Case 27*. A routine chest roentgenogram of an asymptomatic 49-year-old Negro male showed a round lesion in the anterior basal segment of the right lower lobe. Note the presence of a central calcium ring. Innumerable cultures of *H. capsulatum* were seen in the resected lesion. Inset photo of the mass displays concentric layers of fibrous tissue and calcium with a caseated center.

Of the 6 cases, 4 (*Cases 20–23*)[8] showed extensive upper lobe infiltrates with cavitation, bilateral in 3. One of the 4 (the asymptomatic patient) showed thin-walled cavities resembling infected bullae confined mostly to the left upper lobe (figure 12). In the 3 others the cavities were thick-walled and surrounded by dense infiltrate.

Chronic productive phenomena of the disease process and reparative fibrotic

[8] *Case 23* is presented through the kindness of Dr. Herman Nimitz.

changes occasioned contraction in the areas of involvement. In 2 instances of upper lobe fibrocavitary disease marked elevation of one or both hili was observed. In addition, 2 subjects showed a marked shift of the mediastinum toward the side of maximal infiltration. It was not unusual for these lesions to remain stationary over long periods of time; in one instance, for two years.

One patient developed a pneumothorax and subsequently a bronchopleural fistula (*Case 20*). Although there was evidence of dense pleural adhesions in the left costophrenic angle in one case, the presence of a pleural exudate was not observed in any of these patients.

Extensive increased perivascular and peribronchial markings, as the predominant finding, were observed in 3 of the 6 cases. One of these (*Case 24*) presented buccal lesions of histoplasmic origin. In another (*Case 25*) the heavy markings were associated with a round, discrete calcified density with a peripheral band of lesser density, probably representing a histoplasmic granuloma in the right lower lung. This patient has been repeatedly admitted to the hospital in the past four years for "bronchopneumonia superimposed on bronchial asthma and bronchiectasis." The contrast here between this relatively nonspecific change and the active clinical symptoms of chronic respiratory disease is impressive. Peribronchial infiltration is evidently common in histoplasmosis and has often been noted in the *clinical reviews* as being the essential, observable roentgenographic abnormality in a large number of cases. Indeed, similar changes were described by one writer (13) as "strandlike infiltrations in both apical and subapical regions."

Round lesions: Of the 3 males in their fifth and sixth decades presenting round foci (*Cases 26* to *28*), 2 were devoid of symptomatology while one was symptomatic. The latter, a 55-year-old male, had a history of dry nonproductive cough of five months, night sweats, and weight loss. All round lesions showed a central calcific deposit, one of annular form, two with a central dense area surrounded by a peripheral soft tissue density (figure 13). On surgical resection they all lay subpleurally (14), and the firm mass in each case measured from 1.2 to 2.5 cm. in diameter. On microscopy the fungi were morphologically consistent with *H. capsulatum* but showed no growth on culture, indicating that they were probably nonviable. Typically the lesions showed a contiguous gray-white plaque of thickened pleura. Recovery was uneventful following surgery in all instances.

Discussion

A positive histoplasmin skin test was frequently present in several large series of patients who showed miliary intrathoracic calcifications roentgenographically (15–18). In the vast majority, however, it was not possible to distinguish roentgenographically between histoplasmosis and tuberculosis from the character of the calcifications alone. Precalcific lesions in infected children in the endemic region who were sensitive to histoplasmin and not to tuberculin were observed to calcify one to three years and occasionally even a few months after the acute illness; however, in a few instances the calcification was delayed as long as six years (19). In more than one-third of the children whose lesions were progressive over several years, new infiltrates arose as the older ones became calcified.

A roentgenographic finding which has been considered as characteristic of histoplasmic infection is disseminated intrathoracic calcification (20). In endemic areas, 93.5 per cent of such patients show positive histoplasmin skin tests, and a similar relationship exists also in nonendemic areas (7). However, large pulmonary calcified foci and "typical" splenic calcifications are also now to be considered just as specific (10, 11, 21).

In 3 cases (*Cases 3*, *4*, and *16*), children presented here with miliary and multiple nodular calcifications, the period from the initial clinical infection to the definite observation of calcification ranged up to three and four years. In one child (*Case 2*), with a single round calcific lesion following an atelectatic pneumonia, it was four years. The present writers were fortunately able to observe the increasing amounts of calcium deposition in the multiple discrete lesions proceeding from the center of the focus, the intensity of calcification increasing with age.

Prior to the advent of chemotherapy for tuberculosis, miliary dissemination of tuberculous infection rarely, if ever, healed so that calcified miliary lesions can be almost always ascribed to histoplasmic origin (22).

Two of the cases (*Cases 3* and *4*) of miliary calcification, with a history of clinical illness, appear to be a transitional bridge between those fatal cases of disseminated pulmonary histoplasmosis with systemic involvement and the other far more commonly observed roentgenographic entity of multiple disseminated calcific densities in persons without any apparent past history of clinical pulmonary infection.

The roentgenographic evidence of a healed primary complex in histoplasmosis often displays a disparity in size between the peripheral and mediastinal lesion. In *Case 1*, a rounded peripheral calcium density of 5 mm. was observed in the parenchyma; the central component was a massive aggregate of calcified lymph nodes measuring 3.0 cm. in length. In a study of 105 consecutive autopsies in Cincinnati, the overwhelming majority (80 per cent) of calcified intrathoracic foci of histoplasmic origin were 4 mm. and larger; all of those larger than 8 mm. were exclusively of histoplasmic etiology (21). Again, another case (*Case 2*) showed a large rounded parenchymal focus with a central area of calcification and a periphery of soft tissue density. The hilar component of this primary complex was not striking in size. Five of the present cases exemplified the "halo-type" of discrete round focus in children and adults. The large "mulberry-shaped" pulmonary calcification is noted very often in histoplasmosis (21).

It is of interest that all of these lesions showed calcium density within them on routine roentgenographic, laminagraphic, and pathologic examination. The histoplasmoma is similar in appearance to that of a round lesion due to any other chronic infection such as coccidioidomycosis or tuberculosis (23). Its importance in the differential diagnostic consideration of a peripheral bronchogenic carcinoma, which can resemble this lesion, should be emphasized here.

In the 3 cases serving as examples of healed miliary calcification, there was moderate hilar lymphadenopathy at the time of acute clinical infection. However,

hilar lymph node enlargement was relatively more prominent in almost all cases of acute disseminated histoplasmosis with intrathoracic lesions, terminating fatally in infants and children (*Cases 12* to *15*), and in one young adult (*Case 17*). Nevertheless, in most cases of benign active histoplasmosis, the hilar adenopathy was conspiculously present. The active primary complex can present itself with hilar or mediastinal adenopathy dominating the roentgenographic picture without a discernible parenchymal lesion. In one of the adult patients (*Case 10*), hilar adenopathy persisted for as long as seven months. A scalene node biopsy showed a noncaseating granuloma suggesting sarcoid; no organisms were isolated. The question of the relationship of sarcoid to histoplasmosis has been discussed by several observers, there being obvious points of resemblance between the two (5).

It should be mentioned here that the converse was not observed in the present material, that is, the occurrence of a parenchymal infiltrate without mediastinal lymphadenomegaly. This was true in the benign active cases as well as in the fulminating disseminated disease. Indeed, it may be noted that hilar adenopathy of a degree sufficient to produce atelectasis and cough has been reported in children repeatedly negative to tuberculin but who develop positive histoplasmin sensitivity (7).

In the presence of unilateral or bilateral parenchymal infiltrates, one child (*Case 7*) showed a dense infiltrate of the right middle lobe. These infiltrates may easily be mistaken for "unresolved" pneumonia. Three months elapsed before the right middle lobe infiltrate was resorbed (24). The small focal infiltrate seen in the young adults (*Cases 6*, *8*, and *9*) was not found among the children and has been reported as a relatively infrequent form of infiltrate in children sensitive to histoplasmin (19).

Segmental pulmonary involvement along with miliary lesions were also observed in one child with acute disseminated disease (*Case 14*).

It is apparent from the above, however, that no prognostic significance may be attached to the roentgenographic character or appearance of the intrathoracic infiltrates. These show many points of similarity while the disease pursues in quite variable fashion either a fatal or a benign clinical course (25, 26).[9] A nonfatal case (*Case 16*) is of great interest in that the child presented disseminated densities in both lungs; nevertheless, the child recovered after a short, febrile illness. It is emphasized that in all of the other fulminating disseminated cases an impressive splenic and hepatic enlargement was seen on the roentgenogram. In the case in question, however, only the liver shadow appeared enlarged while the spleen appeared normal. After three years, conclusive roentgenographic evidence of disseminated calcifications appeared. This case would then represent a transitional one between patients with symptomatic miliary dissemination and a relatively benign clinical course and the others with a fatal outcome.

Acute widely disseminated histoplasmosis is most commonly encountered in infants and children, occasionally in the young adult (8, 24, 27) and reportedly in

[9] This same extreme variability in prognosis has been observed in adults presenting quite similar intrathoracic forms of the disease (25).

the aged (4). Again, it is considered to be rather frequent in those debilitated by lymphomata or other malignant diseases, or it may complicate tuberculosis. Acute miliary pneumonitis observed in some thirty epidemics shows certain points of resemblance to acute disseminated histoplasmosis (28, 29). Chest roentgenograms of twelve persons involved in the Cincinnati epidemic showed diffuse, finely granular infiltrates in both lungs. Roentgenographic studies after eighteen months did not reveal development of pulmonary calcification. Eight to ten years later, only one of five persons examined showed questionable evidence of calcification in the persisting disseminated nodules (30). However, in almost all of the epidemics reported up to 1953, pulmonary calcification, especially of the disseminated miliary type, has been demonstrated (29).

Of the 6 cases of acute disseminated histoplasmosis in children with intrathoracic findings, 5 were fatal in contrast to the favorable outcome of the epidemic form in adults (29, 31, 32). However, as already observed above, an extreme variability of clinical prognosis prevails with apparently similar intrathoracic lesions pursuing benign or fatal courses.

The occurrence of a pleural effusion in the present series was an unusual event and took place in only one patient, a 17-year-old girl with acute disseminated disease; there was no roentgenographic evidence of the pleural exudate. In the fluid obtained by thoracentesis, *H. capsulatum* was seen on smear and culture. Necropsy revealed 700 ml. of exudate in each pleural cavity. Pleural effusion is, however, reported infrequently by a few writers (5, 16, 33). Puckett, in sixty-seven cases in which pulmonary tissue was resected, observed a sterile pleural effusion in one patient with a positive tuberculin test. Puckett expressed his uncertainty whether the effusion was due to histoplasmosis (14). On the other hand, in the present series in 3 of 4 patients with chronic progressive histoplasmosis demonstrating cavitation, a dense pleural reaction or opacification was noted contiguous to the area involved.

Fibrocavitary histoplasmosis has been shown to be relatively more common in elderly males (12), although one case was reported in a 27-year-old male (34). In the large majority of cases the lesions are found in the upper lung fields, usually with bilateral cavitation. Every patient, therefore, with cavitary involvement of the pulmonary parenchyma in whom no acid-fast bacilli can be demonstrated should be investigated for *H. capsulatum*. It is interesting to note that a review of the literature revealed forty-three cases of cavitary histoplasmosis proved by culture (11–13, 25, 34–39, 40–42); these were associated with tuberculosis in four instances (35, 38, 42, 43).

It should be emphasized that other fungi also produce cavities (*Coccidioides immitis*, rarely Aspergillus or Cryptococcus) and that there are no definite roentgenographic criteria enabling one to differentiate between the cavitary lesions produced by these fungi, the tubercle bacillus, or *H. capsulatum*. It is of clinical diagnostic importance to remember that a negative PPD in the presence of a positive histoplasmin skin reaction should strongly suggest histoplasmosis. In the cavitary cases, as a rule, large quantities of organisms are discharged. Histoplasma can be recovered if searched for diligently.

Summary

Twenty-eight cases of histoplasmosis have been presented with special emphasis on the varied roentgenographic appearance of the disease. Massive intrathoracic calcifications are more typical of infection with Histoplasma than tubercle bacilli. The round lesions or granulomas of histoplasmosis contained calcium density in all of the cases. When calcification is present, it is an important diagnostic consideration in a person from the endemic area.

In 2 cases, both children, a diffuse infiltrate involving a pulmonary segment was observed. One infection pursued a benign clinical course; the other terminated fatally. The delayed process of clearing of these infiltrates, in one case up to four months, suggested unresolved pneumonia as well as tuberculosis.

The characteristic roentgenographic appearance of the primary infection in the young adults in the present series was that of a small peripheral infiltrate. This was either resorbed after a few or several months or went on to calcification, in one such case after one year and two months. Hilar lymphadenopathy, either uni- or bilateral, was always present.

Multiple discrete disseminated calcifications are characteristic scars of previous histoplasmosis. The roentgenographic appearance of histoplasmosis is probably diagnostically specific only in the diffuse miliary, the large intrathoracic foci of calcification, and the "typical" splenic calcifications. In all other instances, e. g., round lesions, isolated infiltrates, disseminated or segmental infiltrates, hilar or mediastinal adenopathy, the diagnosis of histoplasmosis depends on additional evidence (skin tests, complement-fixation tests, and culture or microscopic demonstration of the fungus, *H. capsulatum*). Massive pleural effusion is an unusual event in histoplasmosis. The presence of hepatosplenomegaly in infants and children is to be considered as highly suspicious of histoplasmosis in the endemic area.

Sumario

Patrones Roentgenológicos en la Histoplasmosis

Preséntanse 28 casos de histoplasmosis, recalcando en particular el variado aspecto roentgenográfico de la dolencia. Las calcificaciones intratorácicas masivas son más típicas de infección por histoplasmosis que de tuberculosis. Las lesiones redondas o granulomas de la histoplasmosis contenían condensación de calcio en todos los casos y, si existe, esto sería un importante punto diagnóstico en un individuo procedente de una zona endémica. En 2 enfermos, ambos niños, observóse un infiltrado difuso que comprendía un segmento pulmonar. Un caso siguió una evolución clínica benigna; el otro terminó en la muerte. La demora en el proceso de despejar estos infiltrados, en un caso hasta de cuatro meses, sugería neumonía sin resolución así como tuberculosis.

El típico aspecto radiográfico de la infección primaria en los adultos jóvenes fué, en los pocos casos presentados, de un pequeño infiltrado periférico. Este o bien se reabsorbió al cabo de unos pocos o de varios meses o prosiguió hasta la calcificación, que en uno de esos casos, ocurrió al año y dos meses. Siempre existía linfadenopatía hiliar, ya uni- o bilateral.

Las calcificaciones diseminadas discretas múltiples son típicas cicatrices de histoplasmosis previa. El aspecto radiográfico de las histoplasmosis no es con toda probabilidad específico diagnósticamente más que en la forma granúlica difusa, en los grandes focos intratorácicos de calcificación y las "típicas" calcificaciones esplénicas. En todos los demás

casos, ya sea lesiones redondas, infiltraciones aisladas, infiltraciones diseminadas o segmentales, adenopatía hiliar o mediastínica, el diagnóstico se basa en datos adicionales (cutirreacciones, pruebas de fijación del complemento y descubrimiento cultural o microscópico del hongo *H. capsulatum*).

El derrame pleural masivo es un acontecimiento inusitado en la histoplasmosis. La presencia de hepatoesplenomegalia en lactantes y niños mayores de la zona endémica debe considerarse como sumamente sospechosa de histoplasmosis.

Resume

Aspects radiologiques dans l'histoplasmose

Présentation de 28 cas d'histoplasmose où les auteurs insistent sur les divers aspects de l'affection sur les clichés. Les calcifications intrathoraciques massives sont plus représentatives de l'histoplasmose que de la tuberculose. Dans tous les cas, les foyers arrondis ou le granulome de l'histoplasmose renfermaient des opacités calciques; leur présence doit être considérée dans le diagnostic chez tout individu venant d'une région où l'affection est endémique. Deux patients, tous deux des enfants, présentaient un infiltrat diffus intéressant un segment pulmonaire; chez l'un d'eux, le cours de l'affection fut bénin, chez l'autre, l'évolution fut fatale. La lenteur de la disparition des infiltrats, jusqu'à quatre mois dans un cas, a fait envisager le diagnostic de pneumonie non résolue et de la tuberculose.

L'aspect radiologique caractéristique de la primo-infection chez l'adulte jeune consistait, dans le petit nombre de cas présentés, en un petit infiltrat périphérique. Celui-ci se résorbait en peu de mois ou au bout d'un certain temps, ou bien il subissait une calcification; dans un de ces cas au bout de quatorze mois. La lymphadénopathie hilaire, uni ou bilatérale était toujours présente.

De discrètes calcification multiples représentent les cicatrices caractéristiques d'une histoplasmose antérieure. L'aspect radiologique de l'histoplasmose n'est probablement spécifique du point de vue diagnostique que dans la miliaire diffuse, les grands foyers de calcification intrathoraciques et les calcifications spléniques "spécifiques". Dans tous les autres cas, foyers arrondis, infiltrats isolés, disséminés ou segmentaires, adénopathie hilaire ou médiastinale, le diagnostic repose sur des signes supplémentaires (tests cutanés, réaction de complément-fixation, culture ou visualisation au microscope du fongus *H. capsulatum*. L'épanchement pleural massif est une manifestation inhabituelle dans l'histoplasmose. La présence d'hépato-splénomégalie chez le nourrisson et l'enfant suggère fortement le diagnostic d'histoplasmose dans une région endémique.

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ACUTE RESPIRATORY EFFECTS OF PNEUMOTHORAX IN NORMAL AND VAGOTOMIZED DOGS[1, 2]

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INTRODUCTION

Most reported studies of the physiologic response to lung compression caused by pneumothorax were conducted almost twenty years ago at the time when therapeutic pneumothorax for the treatment of tuberculosis was popular (1–3). Since that era, as therapeutic pneumothorax has declined due to improvements in medical and surgical treatment, the experimental research on pneumotherapy has also waned. In spite of several excellent older reports on studies in patients (4–8) and in animals (9–16), there are still controversial issues about variable results and some unanswered questions about the mechanisms of the response. In particular, variable respiratory results have been obtained because in most instances the pneumothorax volume was not related to lung size or body size and it is difficult to estimate the degree of pneumothorax. Furthermore, much of the work was done in pathologic situations, in which the results were confused by other factors (17). Also the role of reflexes in the physiologic response to penumothorax has not been well elucidated.

The present research was undertaken in order to determine the respiratory response to a standard degree of pneumothorax, namely, insufflation of a pneumothorax air volume equal to the functional residual capacity of the dogs, as recently measured by Harvey and Schilling (18) and Simmons and Hemingway (19). This degree of pneumothorax has been shown by preliminary studies to be adequate to invoke a well-defined respiratory response but not to result in rapid deterioration of the animal. The role of the vagus in this response has been evaluated by conducting the tests on normal and on vagotomized animals.

MATERIAL AND METHOD

Four sets of data were obtained in this study. First, measurements were made on a control group of dogs which were subjected to anesthesia alone. These data were accumulated from 6 dogs followed up to 6 hours after anesthesia; from 8 dogs followed for shorter periods; and from all animals in the experimental groups before they were subjected to vagotomy or pneumothorax. Second, measurements were made on a group of 31 normal anesthetized dogs before and during a 1- to 6-hour period after induction of pneumothorax. Third, the role of the vagus in the respiratory response to pneumothorax was determined on 23 dogs by first performing a bilateral cervical vagotomy and then making measurements before and after pneumothorax. To aid in evaluating this response, a fourth set of data was obtained before and after vagotomy alone in 21 of these animals.

Male and female mongrel fasting dogs weighing between 7 and 30 kg. were anesthetized

[1] From the departments of Physiology and Medicine, University of California Medical Center, and the Veterans Administration Center, Los Angeles, California.

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with intravenous sodium pentobarbital, 25 mg. per kg., and were given additional intravenous or intraperitoneal doses as indicated to maintain light anesthesia. The dogs were tracheotomized and studied in the supine position. After obtaining control data over a period of 30 to 60 minutes, in most cases a volume of air equal to the functional residual capacity was introduced into the pleural space by means of a pneumothorax needle inserted through the fourth or fifth intercostal space in the right lateral chest wall. In one group of 6 dogs on which repeated functional residual capacity determinations were made, the volume of the pneumothorax gas injected was equal to the measured control functional residual capacity. In all others, the average functional residual capacity of 27 ml. per kg. (room temperature and saturation) was introduced over a period of approximately 30 seconds. Post-mortem examination revealed that the air entered both pleural spaces, communicating through the mediastinum.

Data measured or calculated included respiratory rate, tidal volume, minute volume, oxygen consumption, ventilation coefficient $\left(\frac{\text{min. vol., L(BTPS)} \times 100}{\text{O}_2\text{ consumption, ml/min (BTPS)}}\right)$, functional residual capacity (or change in functional residual capacity during pneumothorax induction), oxygen content and saturation, and carbon dioxide content of arterial whole blood or plasma. Both open- and closed-circuit systems (20) were used in making respiratory measurements with a tracheal cannula and a respiratory valve with an instrument dead space of 45 ml. Oxygen content and capacity and carbon dioxide content of arterial whole blood were measured with the Van Slyke-Neill apparatus. Functional residual capacity was determined in duplicate by the standard nitrogen-washout open-circuit method (19) and was corrected for instrument dead space. For these measurements the Lilly nitrogen meter[2] was used to determine nitrogen concentrations of expired gas and of alveolar gas sampled just prior to nitrogen washout and in the last few respirations of 7 minutes of oxygen breathing.

No corrections were made for respiratory nitrogen excretion, since changes in functional residual capacity rather than absolute values were of prime interest in this study. Duplicate determinations of functional residual capacity were made approximately 15 minutes apart, allowing reaccumulation of washed-out nitrogen so that nitrogen excretion would be the same for both runs (21). Duplicate determinations of functional residual capacity differed from the mean of the two by an average of 5.3 per cent of the mean (1.5 ml. per kg.). Immediate changes in functional residual capacity during and following pneumothorax induction were obtained from closed-circuit spirograms.

To evaluate the data, the statistical significance of temporal changes was determined either by comparing mean values for different periods of time, by use of the method of analysis of variance with more than two groups, or by determining whether the slope of the regression line for the measurement plotted against time was significantly different from zero. The method used was the one which appeared most appropriate on visual examination of the plotted data. "Significant results" implies statistical significance, i.e., $p < 0.01$, unless otherwise specified.

Results

Respiratory Rate (table 1, figures 1 and 2)

Normal dogs: The mean respiratory rate for anesthetized dogs before pneumothorax or vagotomy did not change significantly with time over a 6-hour period. On inducing pneumothorax, the respiratory rate increased significantly within one or two respirations to more than double control values and remained essentially unchanged for the 6 hours during which measurements were made.

Vagotomy: Bilateral cervical vagotomy caused the expected decrease in respir-

[2] Waters-Conley Co., Rochester, Minn.

atory rate shortly after vagus section. On initiating a pneumothorax, no further change in respiratory rate occurred, in contrast to the effect in nonvagotomized dogs. In 8 of the dogs in which measurements were made immediately after pneumothorax, slight increases were noted (mean from 9.0 to 10.2); and at a later period some increase was observed for the entire group, but these were not statistically significant.

The increase in respiratory rate on induction of pneumothorax in dogs with intact vagi appears to be reflex, as it occurs too rapidly to be humoral in origin and it is abolished by bilateral cervical vagotomy. The tachypnea is apparently not due to carbon dixoide retention, as a decrease in arterial carbon dioxide occurs after pneumothorax, as will be discussed below. It is also of interest that the tachypnea can be abolished almost immediately by rapid withdrawal of pneumothorax gas.

Tidal Volumes (table 1, figures 1 and 2)

Normal dogs: The mean tidal volume did not change with time after anesthesia alone. After pneumothorax, the control tidal volume decreased about one-third immediately and remained essentially unchanged for three hours. It then rose to levels somewhat above control values between 3 and 6.5 hours.

Vagotomy: Vagotomy alone caused the expected significant increase in mean tidal volume, which nearly doubled during the first hour after vagus section. An increase was noted after vagotomy in 18 of the 19 dogs included in this group. Pneumothorax induction in vagotomized dogs had no effect on tidal volume, either immediately or during the next 2 hours.

It appears therefore that pneumothorax results immediately in rapid, shallow respiration in the normal anesthetized dog. The arguments for the decrease in tidal volume being reflex rather than humoral are the same as for the tachypnea being reflex. It is of interest that respiration does not remain shallow, but that tidal volume eventually increases to a normal level even though the tachypnea persists. This may be due to adaptation in reflexes initiated by the pneumothorax, since it cannot be accounted for by changes in blood gases.

Minute Volume (table 1, figure 2)

Normal dogs: The mean minute volume of respiration before pneumothorax did not change significantly with time up to 6 hours. In the pneumothorax group, the control minute volume almost doubled during the first 15 minutes after induction, remained at this level during the first 3 hours, and rose to more than three times control values between 3 and 6.5 hours. As indicated in table 1, there is considerable variability in these values. However, that a significant increase occurs is attested to by the fact that almost every measurement made after pneumothorax was greater than its control value.

Vagotomy: Vagotomy alone had no significant effect on minute volume. Thus, control of minute volume appears to be related to either blood gases or reflexes not involving the vagi, as contrasted to respiratory rate and tidal volume. Since minute volume remains constant while tidal volume increases, increased alveolar

TABLE 1

RESPIRATORY DATA ON CONTROLS, BEFORE AND AFTER PNEUMOTHORAX, AND BEFORE AND AFTER VAGOTOMY AND PNEUMOTHORAX

	Controls	Pneumothorax					Vagotomy and Pneumothorax				
Respiratory rate (per min.)											
Period (hours)	0–6	—	PNX†	0–0.1	0.1–1	1–4	4–7	—	VAGEX‡	0–1	PNX	0–2
Mean	14	12.4		28.0	26.4	26.0	25.7	18.0		10.4		11.4
S. D.*	7	4.5		6.6	8.8	8.1	11.8	8.3		4.7		4.8
Measurements; dogs	46;25		67;22						39;20		35;20	
p						<0.01				<0.01		>0.05
Tidal volume (ml. per kg.)												
Period (hours)	0–6	—	PNX	0–0.3	0.3–3	3–6.5		—	VAGEX	0–1	PNX	0–2
Mean	17.2	14.8		9.8	9.4	17.1		13.0		24.5		28.4
S. D.	7.4	4.9		1.9	4.4	6.5		3.7		8.0		13.8
Measurements; dogs	46;25		66;24						38;19		35;20	
p						<0.01				<0.01		>0.05
Minute volume (ml. per kg. per min.)												
Period (hours)	0–6	—	PNX	0–0.3	0.3–3	3–6.5		—	VAGEX	0–1	PNX	0–2
Mean	231	158		282	269	490		223		230		265
S.D.	124	58		93	109	260		72		71		89
Measurements; dogs	46;25		56;21						30;20		35;20	
p						<0.01				>0.05		>0.05

Oxygen consumption (ml. per kg. per min.)											
Period (hours)	0–6	—	PNX	0–1	1–3	3–6	—	VAGEX	0–1.5	PNX	0–2
Mean	6.6	5.6		5.8	5.7	7.8	5.3		5.3		5.7
S.D.	1.3	1.6		0.4	1.4	1.2	1.6		1.7		2.4
Measurements; dogs	46;25		63;21					40;20		35;20	
p				>0.05		0.01			>0.05		>0.05
Ventilation coefficient											
Period (hours)	0–6	—	PNX	0–1	1–3	3–6	—	VAGEX	0–1	PNX	0–2
Mean	3.9	3.9		5.8	5.0	6.3	4.6		5.0		5.7
S.D.	1.3	1.7		3.0	2.1	2.6	1.6		2.2		3.9
Measurements; dogs	46;25		61;20					40;20		33;17	
p						<0.01			>0.05		>0.05

* Standard deviation of individual measurements
† Pneumothorax
‡ Vagotomy

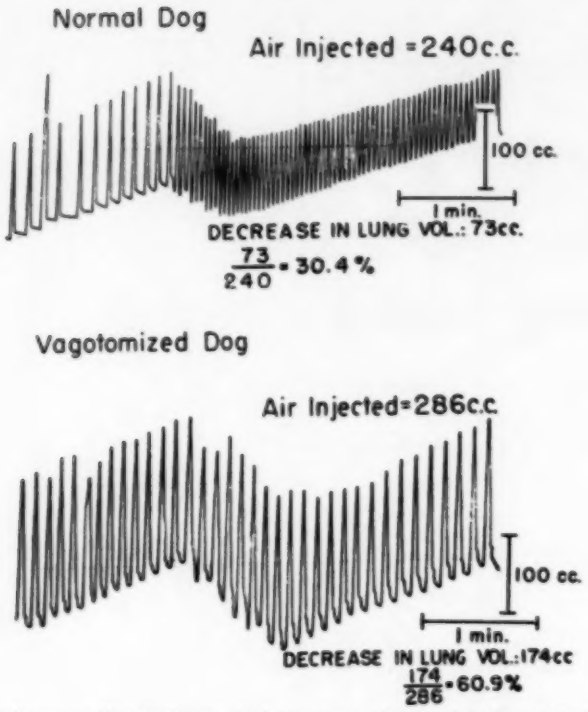

FIG. 1. Spirograms illustrating typical responses of normal and vagotomized dogs to pneumothorax.

ventilation should be expected. That this probably does occur appears to be true since arterial carbon dioxide content decreased, as noted below. However, no direct measurements of alveolar ventilation were made. Pneumothorax induction after vagotomy resulted in a minor increase in minute volume immediately after and during the subsequent 2 hours, but this was not statistically significant.

Thus, an increase in minute volume occurs in normal dogs after pneumothorax, but this response is abolished by vagotomy. This again is consistent with the concept that respiratory changes following pneumothorax are initiated reflexly along pathways including the vagus. As will be demonstrated below, changes in arterial oxygen and carbon dioxide contents or tensions do not initiate the respiratory response following pneumothorax but, rather, are themselves secondary to the reflex respiratory response.

Oxygen Consumption (table 1, figure 2)

Normal dogs: The mean control value did not change significantly over a 6-hour period in a group of anesthetized dogs. The mean control oxygen consumption of 5.6 ml. per min. per kg. for the group of animals subjected to pneumo-

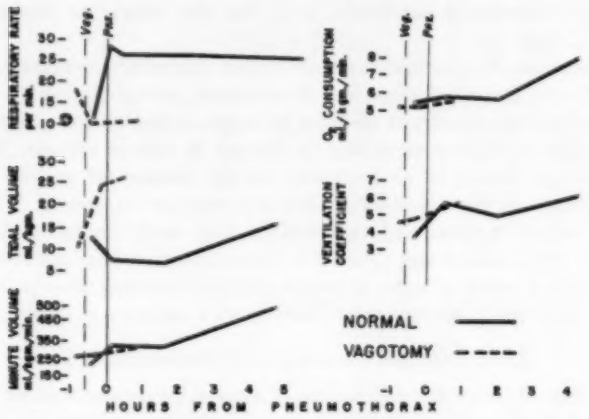

FIG. 2. Graph demonstrating effect of pneumothorax, vagotomy, and vagotomy plus pneumothorax on respiratory measurements. (For means and standard deviations, *see* table 1.)

thorax did not change appreciably during the first 3 hours. (This mean compares with 5.0 ml. per kg. per min. predicted by the formula of Brody for dogs of the same size (22).) However, there was a significant increase during the 3- to 6-hour period.

Vagotomy: Vagotomy alone had no effect on oxygen consumption over a 1.5-hour period thereafter. Pneumothorax induction resulted in a minor increase in oxygen consumption during the first 2 hours after induction, but no significant difference from that of normal dogs was observed. An increase in oxygen consumption has been noted to occur only in the 3- to 6-hour period after pneumothorax in the case of normal dogs. Since measurements were not made on vagotomized dogs during a similar period, no conclusion can be drawn concerning the effect of vagotomy on this late response of oxygen consumption.

Ventilation Coefficient (table 1, figure 2)

Normal dogs: The ventilation coefficient expressed as minute volume (L. per min. BTPS[4]) divided by oxygen consumption (ml. per min. BTPS) times 100 has been described as a measure of ventilatory efficiency (23). The mean for anesthetized dogs did not change with time. The mean for the pneumothorax group rose about one half during the first hour after induction and remained at essentially this same level up to 6 hours. The mean for all postpneumothorax values was significantly greater than the control. Since there is no significant change with time after induction, the effect is immediate and constant.

Vagotomy: Vagotomy itself had no significant effect on the ventilation coefficient. The pneumothorax vagotomy group exhibited a rise after induction, but

[4] Ambient pressure and saturated with water vapor.

this was not statistically significant, as it was due mainly to disproportionate changes in 2 dogs.

Thus, pneumothorax induction resulted in an immediate increase in the ventilation coefficient in normal dogs, but this response was abolished by vagotomy. Since there were no significant changes in oxygen consumption in either group, the differences are largely ascribable to changes in minute volume. This is consistent with the finding of an increased minute volume for normal dogs after pneumothorax and the abolition of this response by vagotomy. Whether the difference between normal and vagotomized dogs really implies a difference in efficiency in respiration is not clear. It is likely, however, that the rise in normal dogs is largely a result of reflex hyperventilation occurring after pneumothorax induction (*see* below), and in a sense does imply a decrease in efficiency.

Arterial Oxygen Content and Saturation (table 2)

Normal dogs: Twelve determinations of arterial oxygen saturation on 11 unanesthetized dogs averaged 91.7 ± 5.5 per cent. After induction of anesthesia, this dropped slightly, but not significantly, to 90.2 in the control series and to 88.7 in the pneumothorax series. This is comparable to the values for anesthetized dogs given by Lewis and Gorlin (24). In the anesthetized control series there was no significant change with time in the present study. Following pneumothorax, the mean saturations during the first 3 hours did not differ significantly from control values. After 3 hours, however, there was a significant drop to 86.0 per cent. In another group of dogs in which arterial oxygen content only—rather than saturation—was measured, the mean content again was not significantly different from control values during a 2-hour period after pneumothorax.

Since no decrease in arterial oxygen saturation or content occurs in the first 2 to 3 hours after pneumothorax, the increase in minute volume of respiration cannot be explained on the basis of hypoxia. This is borne out by a lack of any significant correlation between paired values for changes in arterial oxygen and minute volume.

Vagotomy: Vagal section alone resulted in a slight increase of mean arterial oxygen content which was not significant. While some contents dropped to as low as 85 per cent of pre-section values, these changes appeared to be spontaneous, as increases of a similar order of magnitude occurred in some cases. In contrast to the minimal change in arterial oxygen content of nonvagotomized dogs after pneumothorax, vagotomized dogs showed a significant drop during the first 2 hours after pneumothorax. The significance of the drop is emphasized by the fact that a decrease was observed in 13 of the 15 dogs studied.

Arterial Carbon Dioxide Content (table 2)

Normal dogs: A significant increase in arterial plasma carbon dioxide content averaging 2.2 volume per cent (or 5.2 ± 1.2 per cent of control values) was noted in each of 5 dogs on which the determination was made before and immediately after anesthesia. This is presumably due to respiratory depression. However, during the first 2 hours after induction of anesthesia, blood carbon

TABLE 2

BLOOD GASES IN ANESTHETIZED CONTROLS, BEFORE AND AFTER PNEUMOTHORAX, AND BEFORE AND AFTER VAGOTOMY AND PNEUMOTHORAX

	Controls		Pneumothorax					Vagotomy and Pneumothorax				
	Before Anesthesia	After Anesthesia										
Arterial oxygen saturation												
Period (hours)	—	0–6	—	PNX†	0–1.5	1.5–3	3–					
Mean	91.7	90.2	88.7		89.0	88.7	86.0					
S.D.*	5.5	3.7	3.5		2.0	1.0	1.8					
Measurements; dogs	12;12	48;22		26;14								
p		>0.05					<0.05					
Arterial oxygen content‡												
Period (hours)			—	PNX	0–2			—	VAGEX§	0–1.5	PNX	0–2
Mean			100		97.1			100		101.5		92.1
S.D.*			—		17.8			—		11.7		9.6
Measurements; dogs				24;12					30;15		30;15	
p					>0.05					>0.05		<0.01
Arterial carbon dioxide content‡												
Period (hours)	—	0–1	—	PNX	0–3			—	VAGEX	0–1.5	PNX	0–2
Mean	100	105.2	100		89.3			100		94.6		93.2
S.D.	—	1.7	—		11.4			—		7.8		4.7
Measurements; dogs	—	5;5		58;23					34;17		30;15	
p		<0.01			<0.01					0.02		>0.05

* Standard deviation of individual measurements.

† Pneumothorax.

‡ Values expressed as per cent of control values. Carbon dioxide values are for both blood and serum

§ Vagotomy

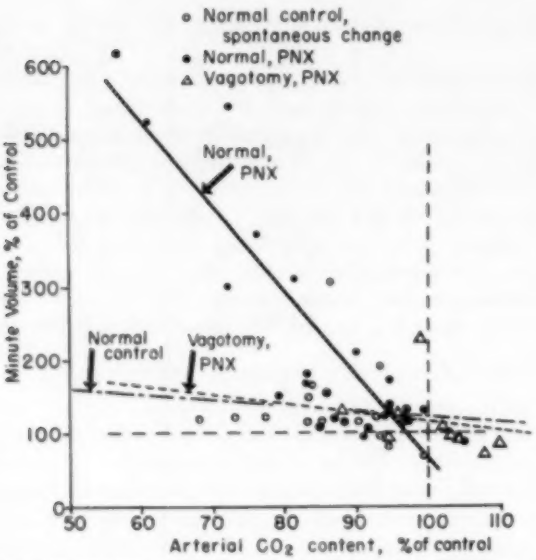

FIG. 3. Graph showing plasma or whole blood carbon dioxide content of arterial blood against minute volume. Points represent simultaneous determinations of the two values: during normal respiration, following pneumothorax in normal dogs, and following pneumothorax in vagotomized dogs, and are expressed as per cent of control values before pneumothorax induction. The lines are regression lines fitted to the points.

dioxide content dropped to 86 per cent of values determined immediately after anesthesia, indicating that this respiratory depression decreases with time. After 2 hours, a relatively steady state appeared to have been reached, since no consistent changes occurred.

Of particular significance is the fact that no significant correlation occurred between spontaneous changes in arterial carbon dioxide content and minute volume, indicating that no causal relationship existed. Determinations of whole blood or plasma carbon dioxide content in a 3-hour period after induction of pneumothorax averaged significantly less than preinduction values. In only 2 of 35 measurements made after initiation of pneumothorax was the plasma carbon dioxide content greater than that before pneumothorax.

Vagotomy: Vagotomy itself resulted in a significant decrease in whole blood carbon dioxide content within the first 1.5 hours. Thirteen of the 17 dogs in which the measurements were made showed a drop, while 2 of the other 4 showed an increase to only 102 per cent of control values. This drop in blood carbon dioxide content is consistent with the decrease in respiratory rate and unchanged minute volume after vagotomy, which implies increased alveolar ventilation in the presence of a constant dead space.[5]

[5] Sevringhaus and Stupfel (25) report an increase in respiratory dead space with bilateral

TABLE 3

EFFECT OF PNEUMOTHORAX* ON FUNCTIONAL RESIDUAL CAPACITY MEASURED BY OPEN-CIRCUIT METHOD

Dog Number	Weight	Control FRC†	Control FRC	Decrease in FRC as Per Cent of Injected Volume	
				0-2 hours	3-6 hours
	kg.	*ml (BTPS)*	*ml/kg.*		
13	16.1	412	25.6	36	32
14	12.0	349	29.1	43	38
15	12.4	318	25.6	56	33
16	7.2	178	24.7	17	31
17	7.8	238	30.5	39	24
18	17.5	462	26.4	8	21
Means			26.9±2.1	33.2±17.7	29.9±6.3

* Volume injected = control functional residual capacity.

† Functional residual capacity.

In contrast to its effect on normal dogs, pneumothorax resulted in only a very slight and insignificant drop in blood carbon dioxide content of vagotomized dogs. This is consistent with the previous observation that the respiratory rate and tidal volume of vagotomized dogs are unaffected by pneumothorax.

In figure 3 are graphically presented all values of minute volume and carbon dioxide determined simultaneously after pneumothorax, both expressed as percentages of prepneumothorax values. It is again apparent from this graph that in almost all cases pneumothorax in dogs with intact vagi results in an increase in minute volume and decreases in carbon dioxide content, and that there is a good inverse relationship between these values. The regression and correlation coefficients for these data are statistically significant. Data for spontaneous changes in repeated measurements made on normal dogs not subjected to pneumothorax showed no significant regression.

In the case of vagotomized dogs there is no apparent relationship between changes in minute volume and changes in carbon dioxide content following pneumothorax. This reflects the fact that marked changes in minute volume after pneumothorax are abolished by vagotomy.

Functional Residual Capacity (FRC) (tables 3 and 4, figures 1 and 4)

Normal dogs: In the first group, the functional residual capacity was measured in duplicate before a pneumothorax equal in volume to the determined FRC was injected. The control FRC values averaged 26.9 ± 2.1 cc. per kg. (uncorrected for nitrogen excretion), which is approximately equal to the mean FRC values determined in a larger series (19) (table 3). In this latter series, it was also demonstrated that no spontaneous consistent changes occur in the functional

vagotomy. In the present studies, this increase appears to be insufficient to overcome the effect of a decreasing respiratory rate (causing increased alveolar ventilation) with constant minute volume.

TABLE 4

EFFECT OF PNEUMOTHORAX* ON FUNCTIONAL RESIDUAL CAPACITY MEASURED BY SPIROMETRY

Normal				Vagotomized			
Dog Number	Volume Injected	Decrease in FRC†	Per Cent Decrease‡	Dog Number	Volume Injected	Decrease in FRC	Per Cent Decrease‡
	ml(BTPS)	*ml(BTPS)*			*ml(BTPS)*	*ml(BTPS)*	
23	243	35	14	24	186	90	48
25	163	55	34	26	248	99	40
28	373	148	40	27	313	240	76
29	189	64	34	30	578	295	51
32	341	163	39	31	351	203	58
33	260	81	31	34	286	174	61
35	240	73	30	36	443	185	42
82	252	100	40	39	286	134	47
83	456	79	16	88	540	249	46
84	456	119	26	90	637	271	43
87	595	208	35	122	797	500	63
91	737	269	36	123	502	352	70
				124	448	253	56
Mean = 31.2 ± 8.3				126	732	400	55
				127	510	266	52
				128	620	321	52
				129	572	376	66
				130	570	332	58
				132	386	210	54
				134	481	321	67
				135	500	288	58
				Mean = 55.4±9.3			

* Volume of pneumothorax = 27 ml. per kg.

† Functional residual capacity

‡ Per cent decrease = "decrease in FRC, ml." divided by "volume injected, ml." times 100

residual capacity of anesthetized dogs with time. During the first 2 hours following pneumothorax, repeat FRC measurements showed a mean decrease of 33.2 per cent of the volume injected (equal to the control FRC). This persisted essentially unchanged up to 6 hours after pneumothorax. These data imply that on introducing a volume of air equal to the FRC, approximately 30 per cent of the injected air is accounted for by lung collapse and 70 per cent by either chest wall expansion or decrease in volume of blood in the thorax. In a second group of normal dogs, the immediate decrease in FRC on injecting 27 cc. per kg. of air (approximately equal to the mean FRC) was determined by the shift in baseline of spirograms (figure 1).[6] The mean decrease was 31.2 per cent of the injected volume, almost the same value obtained by indirect measurement of FRC before and after (table 4).

[6] Increased carbon dioxide excretion resulting from the hyperventilation following pneumothorax will not influence the spirogram, as the change in FRC occurs immediately, while appreciable carbon dioxide excretion takes a matter of minutes (26).

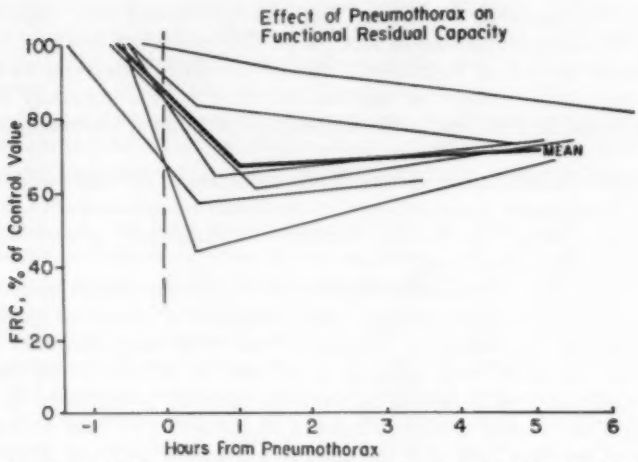

FIG. 4. Graph showing functional residual capacity in a series of 6 dogs before and after pneumothorax, expressed as a percentage of value before pneumothorax. Each value is a mean of two determinations. The volume of air injected was equal to the previous functional respiratory capacity. No correction was made for nitrogen excretion (*see* text).

Vagotomy: Satisfactory spirograms made during bilateral vagus section indicated a significant ($p = 0.02$) mean drop of 5.2 ± 4.3 ml. per kg. after approximately 2 minutes (seven measurements on 7 dogs). This is roughly one-fifth of the control FRC (19). While these changes as measured appear statistically significant, the possibility that they may be partially or totally artifact has not been excluded. In most cases marked hyperventilation occurs during the first 1 to 3 minutes after vagus section. Further, steady state blood carbon dioxide content (*see* above) decreases after vagotomy, suggesting increased alveolar ventilation. The increase in spirometer volume after vagus section could therefore be due to "unloading" of carbon dioxide rather than to a change in the end-expiratory position of the chest wall. Fahri and Rahn (26) report a mean steady state carbon dioxide loss of 1.5 ml. per kg. for each 1 mm. of mercury change in alveolar pCO_2. Assuming an initial pCO_2 of 40 and that the drop in pCO_2 is proportional to the decrease in carbon dioxide content of arterial blood, the steady state pCO_2 change in this study would be approximately $0.06 \times 40 = 2.4$ mm. of mercury (since arterial carbon dioxide decreases about 6 per cent after vagotomy), adequate to account for the apparent decrease in functional residual capacity. During the first 1 to 3 minutes after vagus section, hyperventilation is much more marked than in the steady state, so that the apparent FRC decrease could be greater than that predicted by the calculations. On the other hand, changes in carbon dioxide stores during 2 minutes of constant ventilation are roughly only one-fourth of the steady state changes. It is therefore impossible to evaluate the possibility that the apparent change in FRC is actually due to change in body carbon dioxide stores.

Other data in the literature on the effect of vagotomy on functional residual

capacity are somewhat conflicting. Van Liew (27) found that vagal block by cooling led to a small increase in FRC. Wyss (28) points out, however, that cooling does not affect total vagotomy in that certain fibers responsible for increased inspiratory tonus continue to function and that, in fact, vagotomy leads to a small decrease in FRC due to the abolishment of a minor expiratory-inhibitory reflex.

Spirograms made during pneumothorax induction in 21 vagotomized dogs indicated a significant mean decrease in functional residual capacity of 55.4 per cent of the injected volume. This decrease was significantly greater than the 31 per cent drop in normal dogs.

These data thus indicate that introducing air into the pleural space of normal dogs results in immediate, persistent, and constant lung collapse of approximately 30 per cent of the volume of air injected and chest wall expansion of 70 per cent. That this chest wall expansion is affected by reflexes (in addition to the mechanical factor of increasing intrapleural pressure) is indicated by the fact that vagotomy results in lung collapse of 55 per cent and chest wall expansion of only 45 per cent, and that the difference from the response of normal dogs cannot be accounted for by relatively minor changes in mechanical properties of lung and chest wall caused by vagotomy (27).

Discussion

The acute respiratory response to pneumothorax in the normal anesthetized dog is an immediate onset of rapid, shallow respiration and expansion of the chest wall to accommodate approximately 70 per cent of the volume of gas injected. That these changes are reflex and mediated by vagal afferents is indicated by: (*1*) the rapidity of the onset, which is inconsistent with the response's being humoral; (*2*) the consistent drop in blood or plasma carbon dioxide content without any decrease in oxygen saturation after pneumothorax, indicating that the hyperpnea cannot be explained by blood gas changes but, in fact, actually leads to these changes; and (*3*) the effect of vagotomy, which completely abolishes the response of the respiratory pattern and arterial carbon dioxide to pneumothorax and decreases the compensatory chest wall expansion.

Late changes following pneumothorax include an increase in oxygen consumption and ventilation coefficient, both presumably related to the markedly increased work of ventilation. This increased work in turn is due to an increase in minute volume to levels well above control values. These increased respiratory volumes are in turn associated with much wider swings in intrapleural pressure during the respiratory cycle (12, 29), so that the work of respiration is increased even more than would be expected on the basis of the increased respiratory volumes alone (30). The gradual return of tidal volume to prepneumothorax levels is not explained by the present data, but may be due to adaptation of the reflex leading to the decrease in tidal volume immediately after induction. Since blood carbon dioxide content was not followed over a comparable period, it is possible that the tidal volume increase might be due to carbon dioxide retention resulting from increasingly abnormal ventilation-perfusion relationships in the lung; but this seems unlikely, since a significant decrease in arterial oxygen

saturation did not occur over a prolonged period. Further, the lack of any marked ventilation-perfusion abnormality after pneumothorax has been noted by others (8, 11, 13), although Perkins and associates report such an abnormality after a period of time (31). The return of the tidal volume toward normal is not accompanied by any change in the respiratory rate, and it therefore appears that, if this increase in tidal volume is due to adaptation of a respiratory reflex, then the increased respiratory rate must be mediated by a separate and distinct reflex.

The observation has been made previously that the respiratory response to pneumothorax includes an increase in the respiratory rate and a decrease in the tidal volume in animals (5, 10, 12) and occasionally in man (1, 3). An increase in total minute volume has been noted in both man and experimental animals (1, 3, 6, 9) although no measurements of alveolar ventilation are available.

What appears to be the mechanism of the reflex increase in the respiratory rate was first noted by Breuer (32) who described the increase following chest compression. That this is actually due to a decrease in lung size with a consequent increase in inspiratory tonus has since been determined (28). Christie describes the reflex alteration in respiratory pattern in other terms but on essentially the same basis, ascribing the increased respiratory rate and decreased tidal volume to decreased lung compliance and exaggerated Hering-Breuer reflexes after pneumothorax (12). Acutually, the mechanism for the decrease in the tidal volume is not clear. It could be due to the same "protective" reflex which increases the respiratory rate. On the other hand, this could be ascribed to initial lowering of blood carbon dioxide by tachypnea and a subsequent decrease in tidal volume to maintain alveolar ventilation constant. Nor does the failure of the tidal volume to decrease after pneumothorax in vagotomized dogs shed any light on the mechanism, since the respiratory rate also remains unchanged. This observation is consistent with either mechanism suggested, that is, a reflex decrease or a decrease secondary to an increased respiratory rate.

The effect of pneumothorax on oxygen consumption of humans and experimental animals has been summarized from data published before 1937 by Pinner (1) who concluded that there was either no change or a slight increase. The times at which measurements were made and the temporal relations of the increases were not specified. Chronically, pneumothorax does not appear to affect oxygen consumption in man (3, 4, 6). The data in the present study again indicate no significant effect until more than 3 hours after pneumothorax, when general deterioration occurred, evidenced by decreased oxygen saturation and increased pulmonary arterial pressure (17).

The observation that pneumothorax results in lowering of the arterial carbon dioxide content has been noted in chronic pneumothorax in humans by Richards, Riley, and Hiscock (7), who explained it on the basis of a smaller lung with normal ventilation. A decrease in arterial carbon dioxide with increase in the size of the pneumothorax was also noted in humans by Cournand, Bryan, and Richards (6). Christie and McIntosh, however, observed no such change in humans (5). In the present study, the lowering of arterial carbon dioxide was almost invariable. The determinations were made much sooner after pneumo-

thorax than in the human studies, however, and it was therefore possible to obtain better controls.

The observation that arterial oxygen saturation does not drop after pneumothorax in normal dogs is in agreement with previous observations for dog, rabbit, and man (1, 6, 9–11, 13, 14), except after very large pneumothoraces. As has been mentioned, this has been advanced as evidence that no ventilation-perfusion abnormality occurs and is not unexpected in view of the relatively small amount of lung collapse that takes place. This concept has been verified by the observation that hypoxia, when it does occur, can be corrected by oxygen breathing and therefore must be due to hypoventilation (11, 13). Birath supports this view by noting that hydrogen washout is normal following pneumothorax, indicating that the distribution of inspired gas remains normal (8).

On the other hand, vagotomized dogs must develop either hypoventilation or a ventilation-perfusion imbalance after pneumothorax, since some decrease in arterial oxygen content occurs. That it is not hypoventilation is indicated by the lack of an increase in arterial carbon dioxide. Ascribing the decreased oxygen content to ventilation-perfusion abnormality appears reasonable in view of the greater degree of lung collapse in vagotomized dogs. Alterations in diffusion capacity for oxygen could lead to the same result, but this appears unlikely in an acute experiment, unless one considers a decrease in diffusing area such a change. This can also be described, however, as an alteration in ventilation-perfusion relationships.

The drop in oxygen saturation of normal dogs after 3 hours of pneumothorax is not explained by these data, but appears to parallel a general deterioration, as evidenced by hemodynamic changes (17). The saturation drop may be due to complete collapse of small areas of lung with shunting of blood through these areas, but may also be due to development of minimal pulmonary edema associated with cardiac failure (17).

The fact that pneumothorax gas is accommodated in the pleural space both by decrease in lung volume and by expansion of the thoracic cage (including the diaphragm) is generally agreed (3). Another possible factor, the displacement of blood from within the thorax, may also account for some of the volume of gas introduced (15), but measurements of the magnitude of this effect have not been made. Rough calculations suggest that this factor could not be the major one. All estimates of the amount of lung collapse to be expected from a given pneumothorax agree, at least as far as the order of magnitude. In humans, the decrease in functional residual capacity has been observed to vary from 30 per cent of the volume of gas injected (7) to approximately 50 per cent (8, 33). In rabbits, the lung collapse again is approximately 50 per cent of the injected volume (9, 15, 16). This is greater than the mean in the present study of 31 per cent for dogs, possibly due to species differences.

The quantitative effect of vagotomy on the relative amounts of lung collapse and chest wall expansion resulting from pneumothorax has an important bearing on the determination of the mechanism of the response. Bücher and Lanz (16) noted, as did the present writers, the greater initial lung collapse in vagotomized dogs as compared with normal dogs. However, the writers failed in the present

study to confirm the gradually increasing lung collapse in normal animals over a 30-minute period after pneumothorax which tended to bring the lung size of normal and vagotomized animals ultimately to nearly the same level. The fact that vagotomy affects the chest wall position after introduction of a pneumothorax implies that some reflex mechanism must be involved in this response. That vagal afferents are important in other aspects of the respiratory response to pneumothorax has already been noted. It therefore appears likely that similar afferents may be important in determining chest wall position and the degree of lung collapse. Theoretically, the greater decrease in lung volume and lesser chest wall expansion after pneumothorax in vagotomized animals could be explained by: (*1*) a change in lung elasticity or compliance due to vagotomy; (*2*) a change in chest wall inspiratory tonus due to vagotomy, i.e., a change in chest wall compliance; or (*3*) loss by vagotomy of a reflex initiated by lung collapse and causing chest wall expansion greater than that expected on a mechanical basis alone. The first of these possibilities has been ruled out by Van Liew (27), who showed that the mechanical properties of the lung itself are not affected by vagotomy. The same writer also presents evidence against the second possibility by demonstrating that changes in chest wall compliance after vagotomy should lead to greater chest wall expansion and less lung collapse following pneumothorax, rather than what is actually observed, if this were the only factor.[7] It seems likely, therefore, that the effect of vagotomy on the response to pneumothorax is to abolish a reflex which operates to keep the lung expanded by increased inspiratory tonus of the chest wall only after the pneumothorax is induced. Considerable evidence for such a reflex in normals is presented in a review by Wyss (28) who describes it as a "defense reaction against complete collapse of the lungs" or as a nosoceptive reflex mediated by vagal afferents more or less specific for combating lung collapse and not operating at normal lung volumes. These were first described by Adrian (34) and later confirmed by others. Evidence against such a concept is presented by Bücher (15), who noted that the widening of the chest after pneumothorax is not affected by hyperventilation apnea and, therefore, probably does not involve the respiratory center and is probably not reflex. However, this experiment was conducted with vagotomized animals in which the afferent part of the reflex arc was eliminated, and the presence of such a reflex could not be ruled out. These data do establish, however, that, if a reflex does account for the chest wall expansion in normal dogs, the arc must include vagal afferents.

Summary

The respiratory response to induction of a large pneumothorax in anesthetized dogs is described. The mechanism of this response was investigated by determining the response in dogs with bilateral cervical vagotomy.

[7] Actually, these data of van Liew cannot be accepted as final in establishing that changes in chest wall compliance do not explain the response of vagotomized animals to pneumothorax. Vagal block was obtained by cooling rather than by vagal section. As Wyss has pointed out (28), cooling does not block some vagal afferents from the lung which may augment inspiratory tonus.

The response in normal dogs consisted of an immediate onset of rapid, shallow respiration, with an increase in minute volume and lowering of the carbon dioxide content of arterial blood, implying hyperventilation. This response was completely abolished by vagotomy, indicating that the response is reflex and is mediated by vagal afferents from the lung.

Arterial oxygen content was initially unaffected by pneumothorax, indicating that no ventilation-perfusion abnormality was produced. A late drop in oxygen saturation was associated with general deterioration. Unsaturation did occur in vagotomized dogs.

Pneumothorax did not affect oxygen consumption until after several hours. The ventilation coefficient increased immediately after pneumothorax and remained elevated.

Functional residual capacity decreased by approximately 30 per cent of the volume of the pneumothorax in normal dogs and 50 per cent in vagotomized dogs. The difference is ascribed to a reflex arc with vagal afferents which compensates for extreme degrees of lung collapse.

Vagotomy itself led to the usual decrease in respiratory rate and increase in tidal volume, with no measurable change in minute volume. An increase in alveolar ventilation was indicated by a decrease in arterial carbon dioxide content, however. No effect was noted on arterial oxygen content, oxygen consumption, or ventilation coefficient. A slight decrease in functional residual capacity was noted after vagotomy.

Sumario

Efectos Respiratorios Agudos del Neumotóraz en los Perros Normales y los Vagotomizados

Descríbese la respuesta respiratoria a la inducción de un neumotórax grande en perros anestesiados. Se investigó el mecanismo de esta respuesta determinando la observada en perros en que se había practicado la vagotomía cervical bilateral.

En los perros normales, la respuesta consistió en la iniciación immediata de una respiración superficial y rápida con aumento del volumen por minuto y baja del contenido de bióxido de carbono en la sangre arterial, denotando hiperventilación. Esta respuesta fué absolutamente abolida por la vagotomía, indicando esto que la respuesta es un reflejo, mediando en ella los aferentes neumogástricos del pulmón.

El contenido arterial de oxígeno no se vió al principio afectado por el neumotórax, indicando esto que no se producía anomalía en la ventilación-perfusión. Una baja tardía de la saturación de oxígeno se asoció con deterioro general. En los perros vagotomizados ocurrió insaturación.

El neumotórax no afectó el consumo de oxígeno sino al cabo de varias horas. El coeficiente de aereación aumentó inmediatamente después del neumotórax y permaneció elevado.

La capacidad residual funcional disminuyó aproximadamente en 30 por ciento del volumen del neumotórax en los perros normales y en 50 por ciento en los vagotomizados. Atribúyese esa diferencia a un arco reflejo con los aferentes neumogástricos que compensa los grados extremados de aplastamiento pulmonar.

La vagotomía misma condujo a la habitual disminución de la frecuencia respiratoria y al aumento del volumen corriente sin alteración mensurable del volumen por minuto. Indicó, sin embargo, aumento de la ventilación alveolar una disminución del contenido arterial de bióxido de carbono. No se notó efecto alguno sobre el contenido arterial de oxígeno, el consumo de oxígeno o el coeficiente de aereación. Después de la vagotomía, se notó leve disminución de la capacidad residual functional.

Resume

Effets respiratoires aigus du pneumothorax chez les chiens normaux ou vagotomisés

La réaction respiratoire à la création d'un pneumothorax volumineux chez des chiens anesthétisés est décrite. L'étude du mécanisme de cette réaction a été effectuée par sa détermination chez le chien après une vagotomie cervicale bilatérale.

Chez le chien normal, la réaction consistait dans l'apparition immédiate d'une respiration rapide, superficielle, avec accroissement du volume-minute et réduction de la teneur du sang artériel en CO_2, ce qui implique l'hyperventilation. La vagotomie abolissait complètement cette réaction, ce qui démontre que cette réaction est un réflexe transmis à partir du poumon par des filets centripètes du vague.

Le teneur en oxygène du sang artériel n'était pas initialement affectée par le pneumothorax, ce qui indique qu'il ne se produisait pas d'anomalie dans la ventilation ou perfusion. Une chute ultérieure de la saturation en oxygène s'accompagnait de troubles généraux. Le défaut de saturation se manifestait chez le chien vagotomisé.

Le pneumothorax n'affectait la consommation d'oxygène qu'au bout de plusieurs heures. Le coefficient de ventilation augmentait immédiatement après la création du pneumothorax et demeurait élevé.

La capacité fonctionnelle résiduelle était réduite approximativement de 30% du volume du pneumothorax chez les chiens normaux et de 50% chez les chiens vagotomisés. La différence est attribuée à un réflexe empruntant les filets centripètes du vague et compensant les degrés extrêmes du collapsus pulmonaire.

La vagotomie elle-même entraînait la réduction habituelle de la vitesse des mouvements respiratoires et une augmentation du volume de l'air courant sans modification appréciable du volume-minute. Toutefois, l'accroissement de la ventilation alvéolaire était indiqué par la diminution de la teneur en CO_2 du sang artériel. Il n'a été observé aucun effet sur la teneur en oxygène du sang artériel et sur la consommation d'oxygène ou sur le coefficient de ventilation. Après la vagotomie, une légère diminution de la capacité résiduelle fonctionnelle a été observée.

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THE PREVALENCE OF TUBERCULIN SENSITIVITY AND ITS RELATION TO A BCG VACCINATION PROGRAM AMONG CHINESE IN HONG KONG[1]

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Introduction

Vaccination with BCG is likely to remain a controversial subject for many years to come. Moreover, as the procedure operates in the rather indefinable field of "prevention," it is unlikely that its precise value will ever be ascertained. That BCG has a protective value, however, is well supported by the studies and observations of Rosenthal and associates (1), Ferguson (2), Wallgren (3), Hyge (4), Hertzberg (5), Heimbeck (6), Dahlstrom and Difs (7), Helweg-Larsen (8), Stein and Aronson (9), and others. It is the general conviction of those who have used BCG extensively that it plays some part in the control of tuberculosis, although it is difficult to prove how large a part. From a study of several surveys, Heaf (10) has pointed out that the chances of developing tuberculosis are approximately six times greater in tuberculin-negative persons than in comparable vaccinated ones. The recent early reports from the Medical Research Council of Great Britain (11) are encouraging. The controlled trials undertaken by this Council indicate satisfactorily that vaccination with BCG is efficacious in the prevention of tuberculosis. Their studies, however, are still in progress, and many more important conclusions will undoubtedly be forthcoming.

Tuberculosis constitutes the major public health problem in Hong Kong, where the population borders on three millions. In 1951 the death rate reached a peak level of 208 per 100,000; in 1955 it had fallen to 120 per 100,000.

A mass BCG vaccination campaign was undertaken by the Government Medical Department of Hong Kong in 1952 under the sponsorship of WHO-UNICEF. Local teams were trained by visiting WHO personnel who remained in the colony until the campaign was under way. Although the Medical Department was responsible for the over-all program, the Hong Kong Anti-Tuberculosis Association staffed and maintained one stationary vaccinating center.

The present paper deals only with the work carried out at the clinic operated by the Anti-Tuberculosis Association between May, 1952, and May, 1956. It appears to be an infinitesimal part of the whole program and may be regarded as a by-product of the campaign.

Materials and Methods

All of the vaccination equipment (syringes, needles, PPD, and BCG) was supplied by WHO and was similar to that used elsewhere in its mass campaigns. A single dose of tuberculin, 5 TU, was the only screening test carried out prior to BCG vaccination. The tuberculin was injected intradermally into the middle of the dorsal aspect of the left forearm and

[1] Ruttonjee Sanatorium, Wanchai, Hong Kong.

was calculated by raising a wheal measuring 8 mm. in transverse diameter. An indurated area measuring 5 mm. or more in transverse diameter read three days later was considered "positive." If there was no reaction or if the reaction measured less than 5 mm., the person was vaccinated with BCG at the time of the second visit. The vaccine was the liquid type supplied by the Manila laboratory and was used for as long as six weeks from the date of preparation.

Two dosage systems were in use in the clinic. For infants less than two years old, a single intradermal injection was made in the region of the left deltoid and the dose was estimated by raising a wheal measuring 3 mm. in diameter only. For all those more than two years old, a similar injection was made but the dose was bigger and a wheal raising 6 mm. in transverse diameter was used. The small dose for the infants was chosen as a precautionary measure lest, in the event of complications, the whole campaign would fall into disrepute in the minds of the public.

An attempt was made to carry out a post-vaccinal tuberculin test at ten weeks, one year, two years, and three years, respectively, after vaccination. Again, 5 TU were used and an indurated area measuring 5 mm. or more was considered "positive." The right forearm was used for all post-vaccinal tests.

In addition to the foregoing, which was a routine procedure for all, some special investigations were carried out on smaller numbers. One group of children received a second stronger dose of tuberculin before vaccination. However much to be desired, it was not possible to do this for everyone as it would involve too many visits to the clinic and the people would fail to attend. Groups of children who were considered "negative" after vaccination were also tested with larger doses of tuberculin and the results were compared.

The staff of the clinic consisted of one team—a nurse who gave all the injections, a clerk who kept the records, and a doctor who read the reactions. An attempt was made to ensure as much uniformity of mind as possible in the interpretation of the reactions.

Observations

The reading of one tuberculin test was completed in a total of 70,891 Chinese of all ages. Of this group, 78.55 per cent gave reactions which measured 5 mm. or more in induration and so were considered "positive." Of those more than fifteen years of age, 46,174 completed the tests, and 95 per cent of them gave "positive" reactions. Three thousand, one hundred and ninety-two children less than two years old were tested, and "positive" reactions were found in 6.74 per cent.

From these figures and according to the criteria of positivity employed only 21.45 per cent of the people tested were eligible for BCG vaccination and the vast majority of these were young children.

The level of natural sensitivity to tuberculin among 10,317 persons who attended the clinic consecutively is shown in figure 1. The high percentage in the "negative" segment was due to the fact that about one-third of the group represented in the figure was less than two years of age, in which group only 6 per cent gave evidence of infection. When, however, they are divided into age groups as in figure 2, it may be seen that the percentage in the right-hand section (the presumably infected) increases with each age group as the left-hand section (the presumably noninfected) approaches the baseline. In the age groups two to six and seven to fourteen years, 57 per cent of those who were "positive" gave reactions larger than 15 mm. to the weak dose of 5 TU. When children admitted to Ruttonjee Sanatorium were tested, the majority of whom had

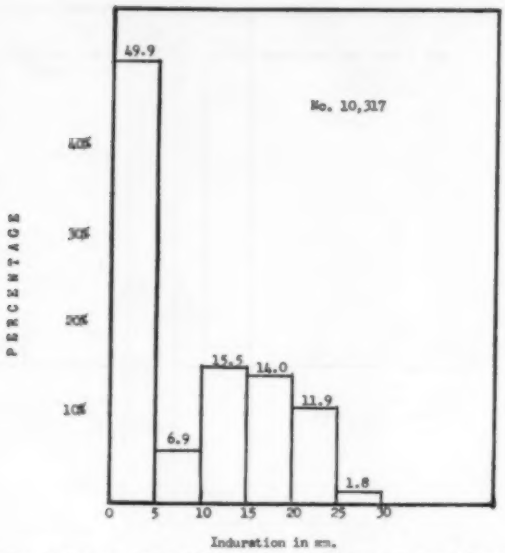

FIG. 1. Distributions by size of reactions to the 5 TU test among 10,317 persons who attended the clinic consecqutively.

"progressive primaries," it was found that 76 per cent gave reactions of 15 mm. or more to 5 TU.

Different Strengths of Tuberculin

A stronger dose of tuberculin was employed in 709 children consecutively who produced no reaction to 5 TU. According to our interpretation, 95 per cent of these children failed to produce a reaction of even 1 mm. to 5 TU. Approximately half of them were given a second test with 10 TU, and the other half a second test with 100 TU. The results are given in figure 3. It was concluded that, for those who were tuberculin negative to 5 TU, the results were substantially the same whether a dose of 5, 10, or 100 TU was used, and that 5 TU were therefore adequate as a screening test for BCG vaccination in Hong Kong.

BCG Vaccination

More than 12,000 persons more than two years of age received BCG vaccination. Ten weeks later 60 per cent of them were rechecked, and a conversion rate of 91 per cent was obtained. The poorest conversion rate was found in the age group from two to six years, in which only 88 per cent produced positive reactions. One year later an attempt was made to carry out another check, but only 50 per cent (3,164) of the original convertors completed the required test, and it was found that an additional 12 per cent had lost their tuberculin sensitivity. Two years later, 1,312 of those who had retained their sensitivity for one year were again checked, and 20 per cent were found to have lost their sensitivity.

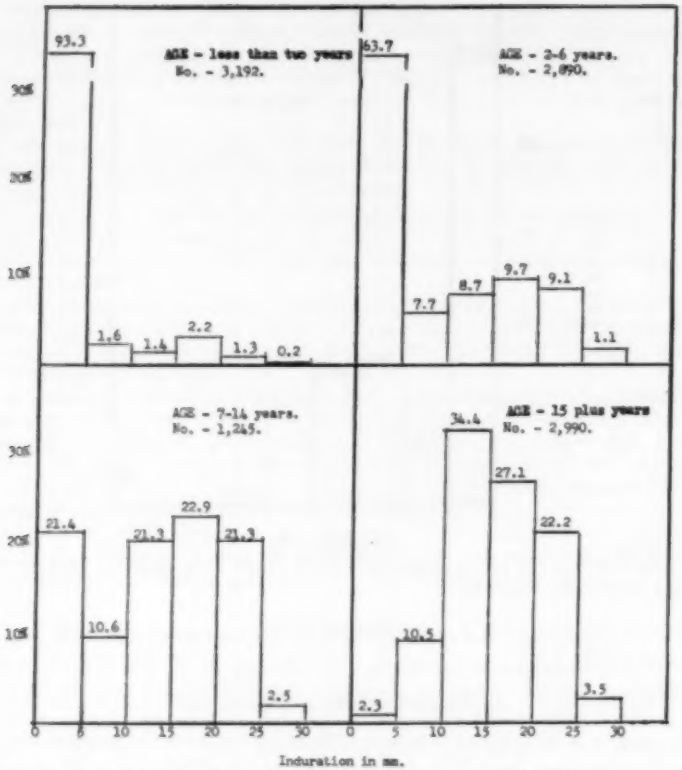

FIG. 2. Distribution by size of reactions to the 5 TU test among the different age groups.

Two thousand nine hundred and seventeen persons less than two years old received BCG vaccination. The dose given them was only about one-third of that given to adults. A conversion rate of 69 per cent only was obtained. One year later, 28 per cent of the convertors had lost their sensitivity.

The level of tuberculin sensitivity obtained after vaccination was much lower than that found in the absence of vaccination. In the age group two to six years, 73 per cent of the tuberculin reactions following BCG vaccination were between 5 and 14 mm. induration. Only 15 per cent gave reactions of 15 mm. or more. In the 2,081 infants who were checked after vaccination, the reactions ranged from 5–14 mm. in 55 per cent, and 14 per cent gave reactions measuring more than 15 mm.

Different Strengths of Tuberculin

Three groups of children who were "negative" to 5 TU after vaccination were given a second tuberculin test. The first group received the test ten weeks after

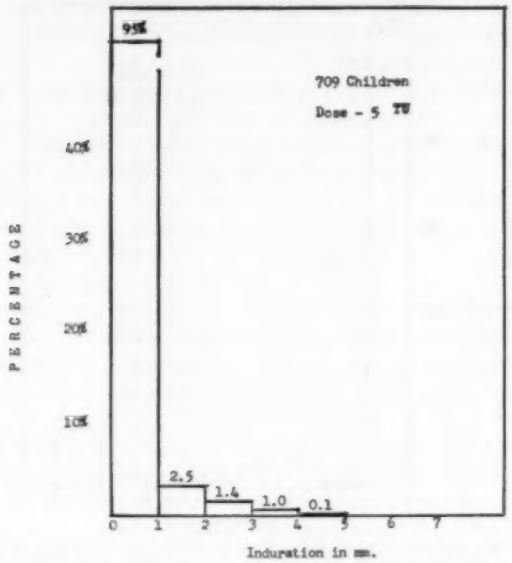

FIG. 3*a*. Frequency distribution of transverse diameter of tuberculin reactions measuring less than 5 mm. in 709 children.

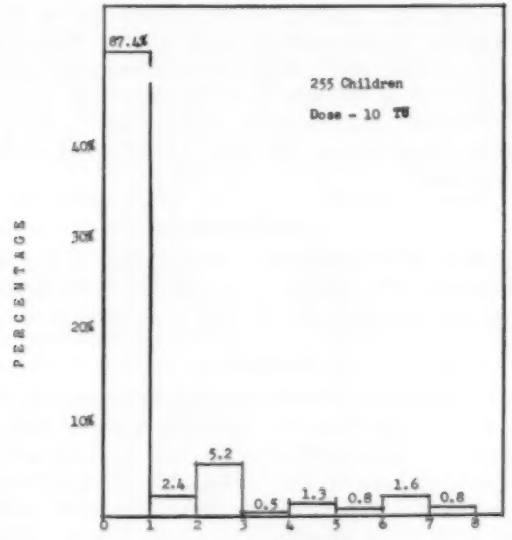

FIG. 3*b*. Frequency distribution of transverse diameter of tuberculin reactions using 10 TU in 255 of the 709 children who reacted with less than 5 mm. to 5 TU (figure 3*a*).

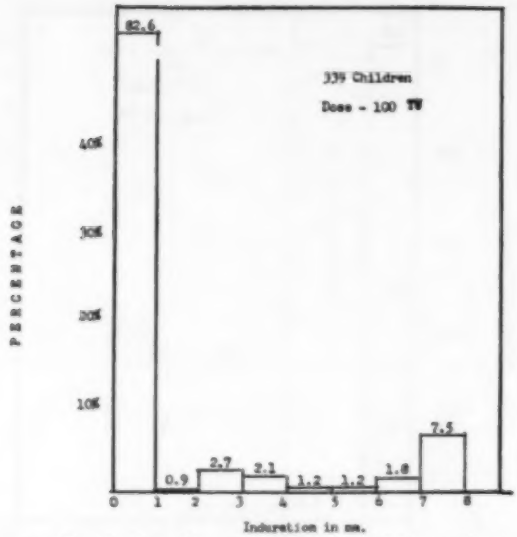

FIG. 3c. Frequency distribution of transverse diameter of tuberculin reactions using 100 TU in 339 children who reacted with less than 5 mm. to 5 TU (figure 3*a*).

vaccination; the second group, one year after vaccination; and the third group, two years after vaccination. In all three groups, more than 90 per cent failed to produce a post-vaccinal reaction of even 1 mm. to 5 TU. When tested with 100 TU, a different picture was found in all groups. With the larger dose of tuberculin, there was a marked shift to the right which indicated that the vaccination had produced some sensitivity which could be detected only by using a strong dose of tuberculin. About 70 per cent of each group moved into the "positive" section when 100 TU were used.

Complications

Complications from BCG vaccination were negligible in this study. Palpable lymph nodes in the left axilla were found in 1 per cent of the infants. The lymph nodes broke down and drained in 0.1 per cent of all vaccinated infants.

DISCUSSION

Assuming that a reaction to tuberculin indicates an infection of the body by tubercle bacilli, it is obvious from the present findings (almost 80 per cent of 70,891 were "positive") that tuberculous infection in Hong kong is very high. In such an area children run a very great risk of becoming infected and of developing disease, and so it is important that they be given whatever protection a vaccination such as BCG can provide. It also seemed in the present study that a very high grade of tuberculin sensitivity existed in Hong Kong. As high as 57 per cent of the children between the ages of two and fourteen years gave reactions

of 15 mm. or more of induration to a dose as weak as 5 TU. The present findings among children suffering from tuberculosis (76 per cent gave reactions of 15 mm. or more) concur with those of Edwards (12) that very few children having a tuberculous infection give small reactions to 5 TU. It is probable that a still smaller dose, i.e., 1 TU, would have been adequate to detect most of the infected children in this clinic, and it would undoubtedly have been wiser to use 1 TU in children suspected of suffering from tuberculosis before using a stronger dose. Palmer (13) found a measurable response to 1 TU in a number of tuberculous patients, but considers it advisable to use a stronger dose among nonreactors. It is accepted, however, that 5 TU are quite appropriate when only a single test is practicable, as was the case in the present clinic.

The studies caried out by Edwards and Palmer (14) have thrown considerable light on tuberculin sensitivity. These writers consider the existence of at least two kinds of sensitivity to tuberculin. That a high-grade sensitivity reflects the incidence of tuberculous infection in a particular geographic area is well recognized, but the significance of "low-grade sensitivity" or the response produced only to a large dose of tuberculin is not well understood. The follow-up studies of the British Medical Research Council trials (11) revealed a higher incidence of tuberculosis developing among those who reacted to a weak dose of tuberculin (5 TU) than among those who reacted only to a strong dose. The British workers do not agree to the entire "nonspecificity" of the reaction brought out by a large dose of tuberculin and not indicated by a small dose.

In the present study it was believed that a very high-grade sensitivity did exist in Hong Kong and that it reflected the incidence of tuberculous infection with reasonable accuracy. The small investigation on the 709 children who were negative to 5 TU and who were tested again with 10 TU or 100 TU supported the view that a low-grade sensitivity did not appear to exist in Hong Kong. The results with the stronger dose of tuberculin were substantially the same as with the weak dose. This led to the belief that 5 TU were adequate for the sorting out of candidates for BCG vaccination in Hong Kong. All through the four years the impression was obtained that prior to vaccination a person was either nonsensitive or undoubtedly sensitive to 5 TU.

Whatever the relationship between tuberculin sensitivity and immunity, the efficacy of a particular BCG vaccine in ordinary practice is widely measured according to its power of producing tuberculin sensitivity.

It is strongly recommended that the fluid vaccine be used in less than two weeks from the date of preparation. Heaf (10) and Van Deinse (15) are particularly emphatic on this point. The low conversion rates in the present study may have been due in a large part to the fact that the vaccine was used for as long as six weeks from the date of preparation, as suggested by the Manila laboratory. The present writer was fully conscious of the deleterious effect of sunshine and skyshine to the vaccine (Edwards and Tolderlund (16)). In spite of the precautions that were taken to protect the vaccine and of the fact that all of the work was done indoors, it is unlikely that the vaccine completely escaped the adverse effects of light. It was unfortunate that for all post-vaccinal

tests the same site was used, and this may have had some bearing on the high reversion rates. Heaf (10) has recently pointed out that the local effects of tuberculin may last for as long as a year. It is also probable that the poor results obtained in infants could be accounted for by the small dose of BCG that was used. A conversion rate of 69 per cent indicated that the dosage must have been inadequate when the same vaccine gave a conversion rate of 91 per cent in the older group (more than two years) in whom more than double the dose was used.

Summary

A summarized version of the work carried out in one BCG vaccinating clinic in Hong Kong during the four-year period, May, 1952–May,1956, is presented. It was part of the WHO-UNICEF-Hong Kong Medical Department mass BCG campaign. A total of 70,891 Chinese of all ages completed one tuberculin test (5 TU). Almost 80 per cent gave "positive" reactions. It was concluded that, if a reaction to tuberculin indicated an infection with tubercle bacilli, then the prevalence of tuberculous infection in Hong Kong must be very high. A small group of children who did not react to 5 TU were tested with 10 TU or 100 TU. The results confirmed the view that a single dose of 5 TU would appear to be adequate as a screening test for BCG vaccination in Hong Kong.

Twelve thousand persons more than two years of age received BCG vaccination. There was a conversion rate of 91 per cent. Of persons less than two years old, 2,917 were vaccinated. The dose for this group was only about one-third of that given to the other group. A conversion rate of 69 per cent was obtained.

Complications from vaccination were negligible. Stronger doses of tuberculin used after vaccination elicited sensitivity when 5 TU failed.

The use of BCG in Hong Kong is limited almost entirely to infants and children, but these are the groups which require the most protection, especially in an environment such as Hong Kong. The vaccine manufactured in Manila is believed to have low potency. This was also the opinion of the workers in Taiwan (17). On the other hand, much more satisfactory and longer-lasting results might have been obtained if an adequate dosage system had been used, especially in infants, and also if the vaccine had been used in less than two weeks from the date of preparation.

Sumario

La Sensibilidad a la Tuberculina y la Vacunación con BCG entre los Chinos de Hong Kong

Preséntase una versión condensada de la labor llevada a cabo en una clínica de vacunación con BCG en Hong Kong durante el cuatrenio de mayo, 1952 a mayo, 1956. Formaba la misma parte de la compaña colectiva con BCG a cargo del Departamento Médico del FINUUM de la OMS. Un total de 70,891 chinos de toda edad completó una prueba con tuberculina (5 UT). Casi 80 por ciento rindieron reacciones "positivas", de lo cual se dedujo que, si una reacción a la tuberculina indicaba infección por bacilos tuberculosos, la frecuencia de la infección tuberculosa en Hong Kong debía ser muy alta. A un pequeño grupo de niños que no reaccionaron a 5 UT se les comprobó con 10 UT o 100 UT. Los resultados confirmaron la opinión de que una sola dosis de 5 UT parece ser adecuada como prueba de tría para la vacunación con BCG en Hong Kong.

Doce mil personas de más de dos años de edad recibieron la vacunación con BCG,

llegando a 91 por ciento la tasa de "viraje" en ellas. De las personas de menos de dos años, se vacunó a 2,917. La dosis para este grupo fué solamente más o menos la tercera parte de la administrada al otro grupo, obteniéndose una tasa de 69 por ciento de viraje.

Las complicaciones de la vacunación fueron menospreciables. Dosis mayores de tuberculina usadas después de la vacunación produjeron sensibilidad cuando fracasaban las 5 UT.

El papel del BCG en Hong Kong se limita casi absolutamente a su empleo en lactantes y niños mayores. La vacuna fabricada en Manila parece ser de poca potencia, opinión esta compartida por los técnicos de Taiwán (20). Por otro lado, probablemente se hubieran obtenido resultados mucho más satisfactorios y duraderos si se hubiese usado un régimen posológico adecuado, sobre todo en los lactantes, y también si se hubiera usado la vacuna en menos de dos semanas desde la fecha de su preparación.

Resume

Sensibilité à la tuberculine et vaccination par le BCG parmi les Chinois à Hong-Kong

Présentation d'un résumé des travaux effectués dans une clinique de vaccination au BCG à Hong-Kong, au cours d'une période de quatre ans, Mai 1952-Mai 1956, au titre de campagne de vaccination collective par le BCG, entreprise par le Service Médical OMS-FISE de Hong-Kong. En tout 70.891 Chinois de tous âges ont été soumis au tuberculine-test (5 UT). Presque 80% d'entre eux présentèrent une réaction positive. D'où il a été conclu que si une réaction à la tuberculine indique l'infection par le BK, la fréquence de la tuberculose doit être très élevée à Hong-Kong. Chez un petit groupe d'enfants qui ne réagissaient pas à 5 UT, le test a été effectué avec 10 ou 100 UT; les résultats ont confirmé l'hypothèse que 5 UT constituaient une dose satisfaisante comme test de dépistage à Hong-Kong.

La vaccination par le BCG a été effectuée chez 12,000 sujets âgés de plus de deux ans; la taux de virage a été de 91%. La vaccination par le BCG a été effectuée chez 2.917 sujets au-dessous de deux ans. Dans ce dernier groupe la dose vaccinale n'était que le tiers de celle de l'autre groupe; la taux de virage n'a été que de 69%.

Les complications de la vaccination furent négligeables. Après échec de la dose de 5 UT, des doses de tuberculine plus élevées ont donné une réaction positive.

A Hong-Kong, l'emploi du BCG est presque entièrement réservé au nourrisson et l'enfant. Ce vaccin, préparé à Manille, paraît posséder une activité réduite. C'est également l'avis de ceux qui l'utilisèrent à Formose (20). D'autre part, des résultats beaucoup plus satisfaisants et plus durables auraient probablement été obtenus si un système de titrage plus adéquat avait été appliqué, en particulier chez le nourrisson, et si le vaccin avait été utilisé moins de deux semaines après sa préparation.

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ANTITUBERCULOUS THERAPY IN MACACA MULATTA MONKEYS[1,2]

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INTRODUCTION

An outbreak of tuberculosis occurred in a colony of approximately 300 *Macaca mulatta* monkeys during the winter of 1952–1953. The colony was destroyed except for 45 animals whose perpetuation was necessary because of their experimental value (1). These monkeys were isolated and received antituberculous therapy. The method of isolation and the results of the initial eighteen months of therapy have been described previously (2). This report concerns the effect of thirty months of antituberculous therapy, followed by a nine-month observation period.

MATERIALS AND METHODS

The physical facilities and isolation procedures of the tuberculous monkey colony were described previously (2). Therapy for tuberculosis was initiated on July 28, 1953, in 45 monkeys, 19 of which were considered tuberculous by means of the intrapalpabral tuberculin test. Koch's Old Type Tuberculin (OT) 0.1 ml. of a 1:10 dilution, was used, and the test was read at forty-eight- and seventy-two-hour intervals. A positive test was indicated by swelling and erythema at the site of injection. The animals received isoniazid[3] orally at the rate of 50 mg. daily (3, 4). Eleven days later this therapy was discontinued and streptomycylidene isonicotinyl hydrazine sulfate[4] therapy was initiated. Each monkey received the equivalent of 125 mg. of streptomycin and 29.5 mg. of isoniazid intramuscularly five times a week (a weekly total of 625 and 147.5 mg., respectively). This therapy was continued through November 2, 1953.

As the clinical condition improved, the therapeutic dosage was reduced in accordance with the antituberculous therapy recommended by Tucker (5). From November 3, 1953, through January 15, 1956, each animal was given the equivalent of 250 mg. of streptomycin and 59 mg. of isoniazid intramuscularly twice each week.

During the thirty months of therapy, one monkey died of tuberculosis three and one-half months after initiation of therapy (Animal *D-4*, table 1); bacteriologic typing proved this to be the human strain of *Mycobacterium tuberculosis*.

Four animals (*A-0*, *X-2*, *Y-1*, *T-6*) died from natural gas poisoning five months after starting the antituberculous therapy. One animal (*Z-6*) died of barbiturate poisoning twenty-one months after the start of therapy. None of these animals displayed gross or microscopic evidence of tuberculosis at necropsy.

Six female monkeys which were tuberculin negative were placed in contact with the monkeys of the tuberculous monkey colony on May 20, 1955. These females did not receive antituberculous therapy prior to, or after, being placed in the colony. On March 27, 1955, an infant was born to the female of the original group. In addition, a male, tuberculin-

[1] From the Radiobiological Laboratory of the University of Texas and the United States Air Force, Austin, Texas.

[2] This study was supported, in part, by funds provided under Contract AF 18(600)-165 with the USAF School of Aviation Medicine, Randolph Air Base, Texas.

[3] Manufactured as Nydrazid by E. R. Squibb and Sons.

[4] Manufactured as Streptohydrazid by Chas. Pfizer & Co., Inc.

TABLE 1

TUBERCULIN TEST REACTIONS OF TUBERCULOUS MONKEY COLONY UTILIZING 0.1 ML., 1:10 DILUTION OF KOCH'S OLD TYPE OF TUBERCULIN INTRAPALPEBRALLY

Animal Number	July 1953	December 1953	March 1954	May 1954	August 1954	November 1954	February 1955	May 1955	August 1955	November 1955	February 1956	March 1956	June 1956	August 1956	October 1956	Death Date	Necropsy Findings
D4																11/ 9/53	Positive for tubercle bacilli
A0																12/27/53	Negative for tubercle bacilli
X2																12/27/53	Negative for tubercle bacilli
Y1																12/27/53	Negative for tubercle bacilli
T6	1	2														12/27/53	Negative for tubercle bacilli
Z6	4	1	1		1	1	2									4/ 5/55	Negative for tubercle bacilli
K8	2	1	1	1	2	1	1	1		2						1/19/56	Negative for tubercle bacilli
X7	2		2	2	2	1	1									5/ 4/56	Negative for tubercle bacilli
A9				1						1						5/ 9/56	Negative for tubercle bacilli
V5						1	1			1		1				5/12/56	Negative for tubercle bacilli
C8	4	3	1	2	2	3	3	2	2	3		3				4/13/56	Negative for tubercle bacilli
A5				1				1			1	1				4/18/56	Negative for tubercle bacilli
Z5	2	2	1	3	3	2	2	1	2	3	2	3				5/21/56	Negative for tubercle bacilli
B7					1	1		1									
D3			1														
W5																	
W7			1														
K9																	
T4																	
U4													1				
V8																	
B5			1							1	1		1				
C1						1	2										
C3										2							
X1								1	2								
Y5					1		1										

K6					1	1				1	1						
Z4			1														
U7			1			1											
B2			2					1					1				
D7	1		1	1	1	1		1		2	1						
W0				1			1	2	1	2		1	1				
A4	2		1			1											
A3	1	1	2		2	2		1	1		1	1					
Y9	1	1	1	2				1	1								
T1	1				1	1	2	1	2	1	2		2		1		
C0													1				
Z0	3		2	1	1	1	2	2	1	1		1					
B8	3	1	1	1	1	2	2		2	1			2		1		
U5	3	1	1	2	2	3	1	2	2		2	1	1		1		
V3	2	2	4	4	3	2	1	2	2	1	2	1	3	1			
U8	3	1	2	3	2	1			1	3	1	2					
Y0	3	1	2			2	2	1	2	1	2	1	2		3		
C4	2	1	3	3	2	2	2	2	1	2	2	3	2	3	3		
C5	3	1	2	1	2	1	1	1	2	3	1	2	2				

CODE: 1—Erythema (considered a suspicious tuberculin reaction).
2—Erythema with tissue swelling (considered a positive tuberculin reaction).
3—Marked erythema with marked tissue swelling (considered a positive tuberculin reaction).
4—Marked erythema with extensive tissue swelling eye closed or ulcerated (positive).
Blank—Negative reaction.

negative monkey escaped from the main laboratory and was incarcerated in the colony in September, 1955. These 8 monkeys comprised a nontreated, tuberculin-negative control group and were maintained in close contact with the monkeys of the tuberculous colony for the duration of the experiment.

Antituberculous therapy was withdrawn from the "treated group" on January 15, 1956. This decision was based on negative tests in the "nontreated group" accomplished every thirty days (with the exception of the pre- and postnatal period) during the first eight months of exposure.

Results

From January 15 to October 15, 1956, no change was noted in the tuberculin reactions of the "nontreated group" of monkeys. During this period of time, 7 animals of the "treated group" died. One animal (*K-8*) died of acute bronchitis, and 6 animals (*X-7*, *A-9*, *V-5*, *C-8*, *A-5*, and *Z-5*) died as a result of vitamin D poisoning. After eleven and one-half months of exposure (three and one-half months after withdrawal of therapy from the "treated group"), one female control monkey died of acute colitis. Of these animals, none displayed gross or microscopic evidence of tuberculosis at necropsy. Bacteriologic cultures of lung, spleen, lymph nodes, and liver were negative. The infant monkey born to the female of the "treated group" was sacrificed in January, 1956 (ten months following birth and thirteen days after withdrawal of therapy from the "treated group") and displayed no gross or microscopic evidence of tuberculosis at necropsy.

The 6 female control monkeys conceived during the period of exposure. Four of the infant monkeys were delivered by caesarean section, the fifth was born prematurely as the result of an abortion at an estimated three and one-half months' gestation, and the sixth was delivered naturally. Four of the live infants were sacrificed at varying ages up to thirty-one days, and the premature infant displayed no gross or microscopic evidence of tuberculosis at necropsy. One infant born normally on April 28, 1956, has not been sacrificed to date.

The reactions to the tuberculin test (table 1) have diminished in intensity during the course of the experiment. This parallels a report of the work at the Medical Biological Station of the Academy of Medicine, U.S.S.R., where monkeys diagnosed tuberculous by tuberculin testing are treated with streptomycin, PAS, and isoniazid Pthivazid® (a hydrazone of isoniazid) until the tuberculin test is negative (6).

The difference in the arithmetic mean body weight of the monkeys diagnosed as tuberculous from the mean weight of the monkeys in the entire group did not vary significantly during the thirty-nine-month course of the experiment; however, weight gain and loss during the experiment fluctuated to some degree (figure 1). Initially, the mean weight of the colony was 3.5 kg. and the weight increased gradually to a peak of 7.2 kg., which occurred seventeen months following initiation of antituberculous therapy. This was followed by a slight recession to a mean of 6.7 kg. with gradual progression to a peak mean of 8.5 kg. (in the twenty-seventh month) and a gradual regression to a low mean of 6.2 kg. (in the thirty-fifth month). This was followed by a gradual increase in mean body weight.

The first depression in body weight is attributable to inclement weather. The

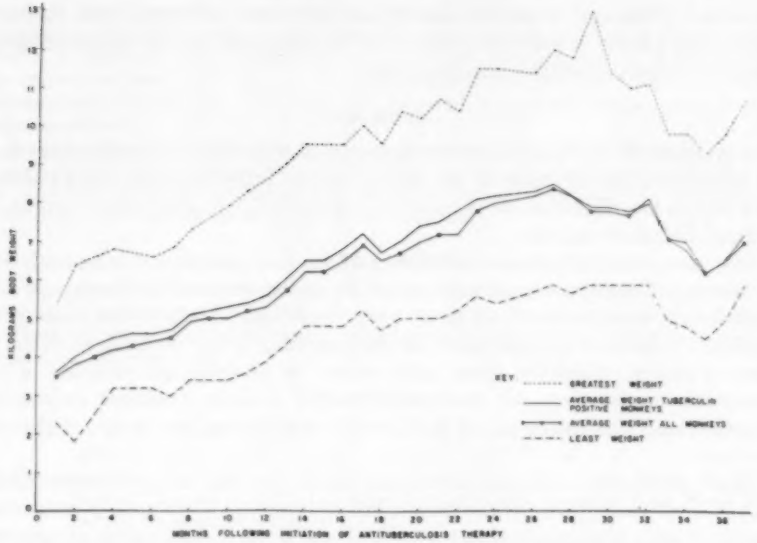

FIG. 1. Average weight in kilograms by month following initiation of antituberculous therapy in a group of 45 *Macaca mulatta* monkeys.

second pronounced depression of the body weight curve was caused by other complicating factors. An outbreak of dysentery in the twenty-seventh month induced the initial part of this weight curve depression, followed in the thirty-second month by vitamin D poisoning. Because of an error on the part of the manufacturer of the food utilized for this colony, 18.3 times the amount of vitamin D specified was incorporated in the diet. This high potency vitamin D diet was fed from February 15, 1956 (thirty-second month) through May 6, 1956, (thirty-fifth month) and resulted in the death of 6 animals mentioned earlier.

DISCUSSION

As has been previously reported by Benson and associates (1), false positive tuberculin reactions in *Macaca mulatta* monkeys have been found to be rare in this laboratory. Of the 15 animals that died or were sacrificed during the course of this experiment, 6 (*T-6*, *Z-6*, *K-8*, *X-7*, *C-8*, and *Z-5*) were considered tuberculous, based on their tuberculin test reactions. These 6 monkeys had, by time of death, received a minimum of five months of antituberculous therapy. None of the 6 displayed gross, microscopic, or bacteriologic evidence of tuberculosis at the time of necropsy.

Ideally, throat swab culturing techniques should have been employed on all animals throughout the course of this experiment; however, facilities were not available to undertake this aspect of study.

The writers are cognizant that the nine-month observation period following

cessation of therapy is not conclusive and are aware of reports that tubercle bacilli may persist in many animal species during and following antituberculous therapy without detectable manifestations.

Summary

A group of 45 *Macaca mulatta* monkeys, 19 of which were clinically diagnosed as tuberculous on the basis of the intrapalpebral tuberculin test, were isolated and received antituberculous therapy (streptomycylidene isonicotinyl hydrazine sulfate) for thirty months.

One animal died of tuberculosis three and one-half months following initiation of therapy. During a nine-month period following cessation of therapy, 9 animals died or were sacrificed. Of these, 2 were nontreated controls and 4 had been clinically diagnosed as tuberculous at the beginning of the experiment. None of these 9 animals displayed gross, microscopic, or bacteriologic evidence of tuberculosis at necropsy. All nontreated control animals displayed exclusively negative reactions to the monthly tuberculin test during the course of this experiment.

Based on clinical evidence and necropsy techniques that included gross, microscopic, and bacteriologic examination, the duration and selection of antituberculous therapy have resulted in the control and possible elimination of tuberculosis in a colony of *Macaca mulatta* monkeys.

Sumario

Terapéutica Antituberculosa en los Monos Macacos Mulatos

Un grupo de 45 monos *Macaca mulatta*, 19 de los cuales habían sido diagnosticados clínicamente como tuberculosos a base de la reacción intrapalpebral con tuberculina, fué aislado, recibiendo entonces terapéutica antituberculosa (sulfato de hidracina isonicotinílica estreptomicilidénica) durante treinta meses.

Un animal falleció de tuberculosis a los tres meses y medio de iniciarse la terapéutica. Durante un período de nueve meses consecutivo al cese de la terapéutica, murieron o se sacrificaron 9 animales. De ellos, 2 eran testigos no tratados y 4 habían sido diagnosticados clínicamente como tuberculosos al comenzar el experimento. De estos 9 animales, ninguno mostró en la autopsia signos macroscópicos, microscópicos o bacteriológicos de tuberculosis. Todos los testigos no tratados revelaton exclusivamente reacciones negativas a las pruebas mensuales con tuberculina durante el transcurso del experimento.

A base de los signos clínicos y las técnicas autópsicas que comprendieron exámenes macroscópicos, microscópicos y bacteriológicos, la duración y selección de la terapéutica antituberculosa ha dado por resultado el dominio y la posible eliminación de la tuberculosis en una colonia de monos *Macaca mulatta*.

Resume

Thérapeutique antituberculeuse chez des singes Macaca mulatta

Un groupe de 45 singes *Macaca mulatta*, comprenant 19 singes pour lesquels le diagnostic de tuberculose pulmonaire avait été fondé sur le test intrapalpébral à la tuberculine, a été isolé et a été soumis pendant trente mois au traitement antituberculeux (streptomycylidène isonicotinyl hydrazine sulfate).

Un animal est mort de tuberculose trois mois et demi après l'instauration du traitement. Durant une période de neuf mois consécutive à la cessation du traitement, 9 animaux sont

morts ou ont été sacrifiés. Parmi ceux-ci 2 étaient des témoins non traités et 4 étaient considérés tuberculeux sur la base des signes cliniques présents au début de l'expérimentation. L'autopsie n'a révélé aucun signe macroscopique, microscopique ou bactériologique de tuberculose chez ces 9 animaux. Tous les animaux témoins non traités présentaient exclusivement des réactions négatives à tous les tests mensuels effectués au cours de cette expérimentation.

En se fondant sur les signes cliniques et les protocoles d'autopsie comprenant l'examen macroscopique, microscopique, et bactériologique, on constate que la durée et la sélection de la thérapeutique antituberculeuse a permis de combattre et peut-être d'éliminer la tuberculose dans une colonie de singes *Macaca mulatta*.

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SOME RELATIONSHIPS BETWEEN PERSONALITY AND BEHAVIOR IN HOSPITALIZED TUBERCULOUS PATIENTS[1]

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Introduction

A consensus among contemporary students of the psychologic aspects of tuberculosis is that the patient's reactions to, and ways of coping with, the unique life situation encountered during treatment in a tuberculosis hospital are determined to a considerable extent by characteristic patterns of psychologic needs and defenses developed by him during his lifetime (1–4). The present study is an attempt to identify and validate several dimensions of personality which, measured early in hospitalization, could contribute to an anticipation and understanding of medically significant behavior patterns during the subsequent course of hospitalization.

On the basis of experimental and clinical work with the Madison Sentence Completion Form (5) and on the basis of reports in the literature, hypotheses were formulated concerning the nature of several personality dimensions which seemed significant to the understanding and prediction of medically important aspects of patients' hospital behavior. The personality variables are described below, with sample responses from sentence completion protocols to illustrate the type of verbal behavior from which these variables were inferred:

Personality Variables and Responses

Dimension A: Characteristic ways of relating to environment

1. Dependency: The patient manifests need to lean on his environment.
   Example: "My cure depends on the doctors and nurses."
2. Regulation: The patient manifests need to have his relationship to environment governed by well-defined rules and norms.
   Example: "My cure depends on my cooperating with the rules of treatment."
3. Self-assertion: The patient manifests need to be independent of the environment.
   Example: "My cure is in my own hands."

Dimension B: Perceived source of initiative

1. Active: The patient perceives himself as the source of initiative in decisions, actions, et cetera.
   Example: "I don't think the treatment is any good."

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2. Acted upon: The patient perceives the source of initiative as coming from the environment and acting on him.
   Example: "They aren't giving me enough attention."

Dimension C: Environment valence

1. Positive: The patient perceives the environment as being friendly and/or permissive.
   Example: "The nurses are like mothers to me."
2. Negative: The patient perceives the environment as being unfriendly and/or restrictive.
   Example: "The nurses are a bunch of nags."

Dimension D: Reactivity level

1. Over-reactive: The patient's reaction to a given situation is more intense than normal.
   Example: "Finding I had TB hit me like an atom bomb explosion."
2. Normal reaction: The patient's reaction to a given situation is within normal range of intensity.
   Example: "Finding I had TB came as a bad shock."
3. Under-reactive: The patient's reaction to a given situation is less intense than normal.
   Example: "Finding I had TB didn't bother me."

The areas of patient behavior selected for study of their relationship to the above personality variables were: (*1*) ward behavior, (*2*) medical progress, and (*3*) mode of termination of hospitalization.

Specific predictions about the anticipated relationships between the personality variables and the behavior areas were made on the basis of clinical observation of the patient population at the Veterans Administration Hospital, Madison, Wisconsin, supplemented by clinical data and experimental findings reported in the literature. The predictions are described below:

(*1*) *Ward behavior*

P-1: Patients who are rated as displaying good ward behavior will, at the time of admission testing, differ on the *A* dimension from patients who are rated as displaying poor ward behavior. The "good" patients will score higher on dependency and lower on self-assertiveness than the "poor" patients.

P-2: Patients who are rated as displaying good ward behavior will, at the time of admission testing, differ on the *C* dimension from patients who are rated as displaying poor ward behavior. The "good" patients will perceive their environment as being more friendly than do the "poor" patients.

P-3: Patients who are rated as displaying good ward behavior will, at the time of admission testing, differ on the *D* dimension from patients rated as displaying poor ward behavior.

(*2*) *Medical progress*

P-1: Patients who are rated as evidencing satisfactory medical progress will, at the time of admission testing, differ on the *A* dimension from patients who are rated as evidencing unsatisfactory medical progress.

P-2: Patients who are rated as evidencing satisfactory medical progress will,

at the time of admission testing, differ on the *D* dimension from patients rated as evidencing unsatisfactory medical progress.

(3) *Termination of treatment*

P-1: Patients who voluntarily terminate their hospitalization will, at the time of admission testing, differ on the *A* dimension from patients leaving the hospital with medical sanction. The latter will score higher on dependency and lower on self-assertiveness than the former.

P-2: Patients who voluntarily terminate their hospitalization will, at the time of admission testing, differ on the *B* dimension from patients leaving with medical sanction.

P-3: Patients who voluntarily terminate their hospitalization will, at the time of admission testing, differ on the *C* dimension from patients who leave with medical sanction. The latter will perceive their environment as being more friendly than do the former.

P-4: Patients who voluntarily terminate their hospitalization will, at the time of admission testing, differ on the *D* dimension from patients leaving with medical sanction.

Methods

Assessment of personality: The problem was to select or devise a method of reliably assessing a patient early in hospitalization on each of the four selected personality dimensions. A basis for such assessment existed in the material from which the personality dimensions were originally derived, the verbal productions of patients on the Madison Sentence Completion Form. Stephenson's Q-technique afforded a method whereby these verbalizations could be utilized to yield the desired information in a form amenable to objective scoring and analysis.

The Q-sort calls for the subject to describe himself in terms of a number of statements having to do with beliefs, attitudes, feelings, et cetera, by gradating a number of such statements in terms of how accurately he believes they do or do not describe himself. It is possible then to analyze statistically the results of the sorting (6). The set of items may be selected according to criteria stemming from a particular theory, and this set then represents and defines the concepts used in the theory. In this study the four personality dimensions were defined by psychologic characteristics of the patients' statements of attitudes and feelings toward various aspects of their hospital situation.

A variation of Q-technique useful for theory testing is available in the structured Q-sort. A balanced-block design is utilized wherein each level of each variable appears in combination with each level of the other variables. This variation lends itself to convenient statistical treatment by analysis of variance, and economy of item-population is possible while studying a number of variables simultaneously. Since the present study involves four dimensions of personality which may be arranged factorially, the Q-sort designed was of this type. The model for the Q-sort constructed for the study is as follows:

Dimension	Levels		
A	*a*. Dependency	*b*. Regulation	*c*. Self-assertion
B	*d*. Active	*e*. Acted-upon	
C	*f*. Positive	*g*. Negative	
D	*h*. Over-reactive	*i*. Normal-reactive	*j*. Under-reactive

Utilizing the balanced-block design, this model yields 36 combinations of levels. To get a better sampling of items while retaining practical test size, two replications of each combination were used, giving a total item *N* of 72.

In the construction of the Q-sort, a large number of items from patients' protocols were submitted to five judges who independently rated each item on each of the four personality dimensions. Items receiving less than four-fifths agreement on any one of the four dimensions were discarded, and 72 items judged to be most representative of the dimension combinations were selected for the Q-sort population. The high degree of agreement among the judges suggested that the items were representative of the various dimension combinations, and that construct validity of the test was high. The list of these items follows:

Q-sort Items

adfh[3]	I place complete faith in my doctor.
	I am constantly grateful to people for taking care of me here.
adfi	I'm happy to be here getting help.
	I look forward to mealtimes.
adfj	I like people to be nice to me.
	I kind of like to lie in bed.
adgh	I'm homesick all the time.
	I'm sure I'm not getting all the help I need.
adgi	I often get lonely and want to go home.
	I'm often concerned about myself.
adgj	I feel pretty weak these days.
	I kind of wish people would like me more.
aefh	My doctor takes wonderful care of me.
	The nurse is just like a mother to me.
aefi	My doctor is sympathetic and kind.
	Bed rest makes me feel rested.
aefj	Bed rest makes me nice and drowsy.
	It's not really for a patient to question things.
aegh	Lying here makes me terribly lonely.
	Finding I had TB made me the sickest person in the world.
aegi	My doctor doesn't show enough personal interest in me.
	They should do more to try to satisfy a patient's wants.
aegj	My doctor's visits could be a little longer.
	Some of the people here aren't so nice.
bdfh	It's a crime to let people leave AMA when they can infect others.
	I would never even think of leaving as long as I was a danger to others.
bdfi	The rules are part of the treatment and that's how I look at them.
	I intend to use my time constructively while I am completing my cure.
bdfj	I like to watch and analyze what goes on around here.
	I like to think when lying in bed.
bdgh	I worry constantly about various things while I am here.
	I feel guilty for being such a burden on society.
bdgi	I sometimes worry about my ability to fulfill my responsibilities when I get out.
	I'm afraid that being helpless might make me lose my self confidence.
bdgj	Once in a while I feel a little guilty about lying in bed doing nothing.
	I feel I ought to be doing a little more to better myself while I'm here.
befh	The rules here are excellently planned for the patient's quick recovery.
	The cleanliness techniques of this hospital are tops.
befi	Routines here run pretty efficiently considering everything.
	The staff carries out its duties conscientiously and efficiently on the whole.

[3] Combinations of dimension levels.

befj The rules are planned all right I guess.
I guess things here are run O.K.
begh Having TB makes me feel worthless.
Everything is going wrong at home without me.
begi The staff should be more consistent in enforcing bed-rest schedules.
The doctors could be more conscientious in keeping a patient informed of his progress.
begj Things outside might not get along without me.
It would be nice if things were run a little more orderly.
cdfh I feel full of pep and vigor.
I really enjoy palling around with the guys on the ward.
cdfi I could go for some of the cute nurses around here.
I like to kid around with the nurses.
cdfj I don't worry about having TB.
I took the news of TB in stride.
cdgh I often resent the loss of freedom here.
I sometimes get mad at restrictions and feel like walking out.
cdgj I don't think much one way or the other about the staff.
I don't think I believed them when they told me I had TB.
cefh The guys around here are all swell Joes.
This place is a swell hotel.
cefi The nurses are real pals to a guy.
Pals usually stay pals even when you have TB.
cefj The nurses aren't a bad bunch of kids.
TB won't affect my life much.
cegh This place is like a prison.
Patients aren't allowed to think for themselves here at all.
cegi They try to tie you down too much here.
My doctor doesn't tell me as much as I have a right to know about my case.
cegj Sometimes the nurse gets a little bossy.
They could allow us a little more freedom.

Each subject was asked to force-sort the items of the Q-sort into 11 classes according to the following pattern:

	Agree least—Neutral—Agree most										
Frequency (Forced)	2	4	6	8	10	12	10	8	6	4	2
Classes (Item scores)	0	1	2	3	4	5	6	7	8	9	10

Since it was of interest to evaluate group differences in this study, the analysis used followed the method outlined by Block, Levine, and McNemar (7) which provides a test of group psychometric patterns.

At this point a study was conducted to determine the reliability of the Q-sort and to develop an effective test administration procedure. Fifteen patients were used as subjects. The test was administered individually to each patient and was then readministered after approximately fourteen days. The test-retest correlations for the 15 subjects ranged between 0.65 and 0.88, with a mean r of 0.77.

A simple, step-by-step administration technique was developed for the Q-sort which was effective in avoiding misinterpretation of instructions by the patients, none of the subjects evidencing difficulty in following the procedure outlined below. Little resistance to taking the test was encountered, apparently because it was meaningful to the patients in terms of their life situations.

Q-Sort Administration Procedure

1. Each patient was seen privately in his ward dayroom.
2. Initial orientation was given: "As you know, a patient's feelings and attitudes have

an effect on his medical recovery. Often two equally sick patients will have very different courses of treatment and recovery. We believe that knowing your reactions to the hospital, the docters, et cetera, will help us to better understand your response to treatment and can help us to plan your treatment more effectively. We have developed a method of allowing you to express your feelings and attitudes in areas which are most important to hospitalized tuberculous patients. This test is given to all new patients after they have been here about a month. Do you have any questions to ask?"

3. Initial sorting instructions were given: "We have here 72 cards each with a statement on it reflecting different attitudes about various aspects of your hospital situation. The first thing I would like you to do is to read these statements one at a time and divide them into two groups—those with which you agree and those with which you disagree. After you have done that, we'll continue with the test."
4. After the patient divided the cards, those with which he agreed were spread out before him and further instructions were given: "Now I want you to read through all these cards and choose the two with which you agree most strongly." When this was done, the two cards chosen were placed in the two rectangles at the extreme right of the Q-board (class 10). The patient was then instructed: "Now from the cards still on the table I want you to choose the four with which you agree most strongly." The four cards selected were hung in the four rectangles in class 9. This procedure was continued until all cards on the table were used up.
5. The cards with which the patient disagreed were spread before him: "Now read through these cards and choose the two with which you disagree most strongly." These cards were placed in the rectangles of class 0, and the process was continued until the supply of cards on the table was exhausted. At this point the 72 item-cards were distributed over the 72 positions on the Q-board.
6. The attention of the patient was then directed to the Q-board: "These cards describing attitudes and feelings about the hospital situation are now arranged on this board going from those with which you agree most on the right through those with which you agree least on the left. I'd like you to check the board a last time to make sure that it's arranged the way you want it. Feel free to make any changes you want."

The Q-sort was administered to all patients who were forty-five years of age and less and who were admitted to the Madison Veterans Administration Hospital during a four-month period with a diagnosis of pulmonary tuberculosis. The Q-sort was administered to each patient approximately one month after admission to the hospital, giving him a chance to form and crystallize initial feelings and attitudes about the various aspects of his new life situation. The behavior groups subsequently studied were drawn from these patients.

Assessment of ward behavior: Assessment of ward behavior was accomplished by use of the Ward Behavior Rating Scale (8). The head nurse of each ward rated each subject on her ward, using this scale, approximately three months after his admission to the hospital. This period permitted behavior to be observed, while minimizing the chance that the patient would be transferred from the ward to surgery or to an ambulatory ward before behavior could be rated. The patients rated were arranged in order of their scores, and the distribution was divided into thirds, with the top and bottom thirds designated as the "good" and "bad" groups, respectively. It was believed that this procedure yielded groups comprised of patients whose ward behavior could validly be described as "model" or "problem." The Q-sorts of the patients composing these groups were analyzed to test the predictions made in this area.

Assessment of medical progress: The criteria for rate of medical progress were medical ratings made on the basis of chest roentgenograms which were routinely administered to each patient every two months. A rating index of medical change already in use at the Madison Veterans Administration Hospital was utilized. The reference point taken for rating the degree of medical change was the state of illness at the time of the first roentgenogram. The possible medical change ratings were: marked improvement, moderate improve-

TABLE 1

MEDICAL PROGRESS GROUPS

	N	Average Index of Medical Change*	
		2 Months	4 Months
"Satisfactory" group	12	2.42	1.75
"Unsatisfactory" group	12	3.58	3.33

* These groups were matched for initial diagnosis, the reference point against which medical change was rated.

ment, slight improvement, no change, slightly worse, moderately worse, and markedly worse. After consultation with the physician in charge of research at the Madison Veterans Administration Hospital, the progress rating at the four-month point of hospitalization was selected as an appropriate basis for selecting patients for satisfactory and unsatisfactory medical progress groups. The roentgenograms of all patients were rated at the same time by a single expert. Two groups, each composed of 12 patients who had placed at the extremes of the ratings, were selected on the basis of these ratings (table 1).

Termination of treatment: Although it is easy to determine from medical records a patient's discharge classification, evaluating discharge behavior on this basis is not satisfactory. For example, it is not certain that a patient receiving a maximal hospital benefit discharge within two or three months of admission to the hospital is comparable in personality or behavior with a patient who remains hospitalized for two or three years, although both may be classified as having maximal hospital benefit discharges; nor is it difficult to anticipate differences between patients who leave irregularly within a month or two and those who remain a year or more before leaving in this way. In an attempt to select groups homogeneous in behavior in this area, the criterion adopted was that of self-initiated termination of hospitalization versus medically sanctioned discharge, and an attempt was made to get "pure cultures" in each group. Patients who were selected for the "voluntary" discharge group were those who had records of hospitalization with multiple absences without medical sanction. In like manner, patients selected for the medically sanctioned discharge group had, wherever possible, records of multiple maximal hospital benefit discharges. As there were not enough patients available for the latter group with multiple maximal hospital benefit discharges, the group was filled out with patients who received such discharges from the Madison Veterans Administration Hospital only after prolonged hospitalization. It was hoped by this method to obtain one group made up of patients who repetitively and characteristically terminated their hospitalizations on their own initiative without medical sanction and a second group whose members strongly evidenced the characteristic of remaining hospitalized until medical sanction for leaving was given. Two such groups with 10 patients in each group were selected and their Q-sorts were analyzed to test the predictions made (table 2).

RESULTS AND DISCUSSION

Ward behavior: The results of the analysis of data in the ward behavior area appear in table 3. In table 4, the group mean Q-sort scores for dimensions *A*, *C*, and *D* are shown.

The first prediction in the ward behavior area, that good ward patients would, in terms of their Q-sort performance, tend to relate to their environment with more dependency and less self-assertiveness than the poor ward patients, was confirmed. The second prediction in this area, that good ward patients would perceive their environment as more friendly and permissive than would the poor

TABLE 2

TERMINATION OF TREATMENT GROUPS

	N	Group Averages			
		Average Number of TB Hospitals	Average Number Unsanctioned Discharges	Medical Class* at Discharge	Days in Madison Hospitalization
Maximal hospital benefit group	10	1.7	0	6.4	374
"Voluntary" group	10	3.2	2.6	2.4	217

* Medical classes represent the stages of medical recovery in terms of activities allowed. They range from Class 1, which indicates total bed rest, to Class 7, which is generally the final ambulatory stage before discharge. Class 4 represents the highest point of progress in the bed-rest wards, while Class 5 marks the transition to the ambulatory wards.

TABLE 3

ANALYSIS OF THE PREDICTED GROUP DIFFERENCES IN THE WARD BEHAVIOR AREA

Personality Dimension	F (Test for Significance of Differences between Groups)
A	6.46*
C	4.78†
D	2.32 (not significant)

* Difference between groups was such as would be expected to occur by chance less than one time in one hundred ($p < 0.01$).

† Difference between groups was such as would be expected to occur by chance less than five times in one hundred ($p < 0.05$).

TABLE 4

MEAN SCORES OF WARD BEHAVIOR GROUPS FOR DIMENSIONS A, C, AND D

	Dimension A			Dimension C		Dimension D		
	D	R	SA	P	N	OR	NR	UR
Good behavior group	125	131	104	222	138	118	125	117
Poor behavior group	115	129	116	205	155	112	132	116

ward patients, was confirmed. The third prediction in the area, that good ward patients would differ on the *D* dimension from poor ward patients, was not confirmed.

These findings support clinical consensus in the literature, as well as the few studies in this area, which have tended to assign central importance to dependency–independency conflicts and their resolution as determiners of hospital behavior, while suggesting that the patient's personality is the major determinant of his specific patterns of conflict and modes of resolution. It would seem that the ability to evaluate objectively dependency–independency conflicts early in hospitalization should enhance our ability to evaluate experimentally various methods of dealing preventively with anticipated problem patients. Possible methods might include the controlled use of environmental manipulation, such

TABLE 5

ANALYSIS OF THE PREDICTED GROUP DIFFERENCES IN THE MEDICAL PROGRESS AREA

Personality Dimensions	F (Test for Significance of Differences Between Groups)
A	0.28 (not significant)
D	5.62*

* Difference between groups was such as would be expected to occur by chance less than one time in one hundred ($p < 0.01$).

TABLE 6

MEAN SCORES OF MEDICAL PROGRESS GROUPS FOR DIMENSIONS A AND D

	Dimension A			Dimension D		
	D	R	SA	OR	NR	UR
Satisfactory progress group	120	128	112	115	131	114
Unsatisfactory progress group	117	129	114	125	123	112

as roommate assignment and staff assignment and orientation, as well as more direct approaches by way of individual and group psychotherapy and counseling.

Medical progress: The results of the analysis of data in the medical progress area appear in table 5. In table 6 are shown the group mean Q-sort scores for dimensions *A* and *D*.

The first prediction made in this area, that patients rated as evidencing satisfactory medical progress would differ on the *A* dimension from patients rated as evidencing unsatisfactory medical progress, was not confirmed. The second prediction in this area, that patients evidencing satisfactory medical progress would differ on the *D* dimension from patients evidencing unsatisfactory medical progress, was confirmed. It is indicated in table 6 that the satisfactory progress group evidenced significantly less over-reactivity and higher normal reactivity than the unsatisfactory progress group.

The literature is equivocal with respect to the role of dependency–independency conflicts in affecting medical progress. Some observers describe medically successful patients as those able to accept the dependent role with minimal inner conflict and tension. Others believe that dependent patients often suffer prolonged illness because their basic dependency needs are better gratified in the hospital than outside. The present study does not show the dependency–independency factor, as defined by the Q-sort, to be significantly related to medical progress. However, it does demonstrate a clear relationship between medical progress and the characteristic intensity of the patient's psychological reactions to his environment. The exact nature of the relationship between Q-sort reactivity patterns and somatic functioning is not clear. It is possible to hypothesize that a relationship does exist and that various characteristic psychological patterns of tension handling are systematically related to aspects of somatic functioning, with consequent effects on the course of physical recovery. Experimental studies for the presence and nature of such relationship could be of interest. In addition,

TABLE 7

ANALYSIS OF THE PREDICTED GROUP DIFFERENCES IN THE TERMINATION OF TREATMENT AREA

Personality Dimensions	F (Test for Significance of Differences Between Groups)
A	2.89 (N.S.)
B	0.00 (N.S.)
C	0.49 (N.S.)
D	1.47 (N.S.)

TABLE 8

MEAN SCORES OF TERMINATION OF TREATMENT GROUPS FOR DIMENSIONS A, B, C, AND D

	Dimension A			Dimension B		Dimension C		Dimension D		
	D	R	SA	A	AU	P	N	OR	NR	UR
Maximal hospital benefit group...	118	136	106	190	170	215	145	116	129	115
Voluntary group.....	120	125	115	190	170	209	151	121	123	116

controlled study of the effects of somatic and psychotherapeutic intervention on the medical progress of patients whose psychological reactivity patterns suggest poor medical prognosis would also seem to be of interest and potential worth.

Termination of treatment: The results of the analysis of data for the predicted relationships in this area appear in table 7. The group mean Q-sort scores within the dimensions are shown in table 8.

Inspection of table 10 indicates that none of the predicted group differences in this area reached significance.

This finding was surprising, as much of the preliminary work with the Q-sort was carried on with known maximal hospital benefit and unsanctioned discharge groups, and the predictions made in this area were based on extensive preliminary observations. A posteriori examination of the data suggested additional hypotheses about the nature of the psychological dynamics underlying the various forms of unsanctioned discharge behavior. The "voluntary" discharge group used in the experiment consisted of 10 subjects, 6 of whom had left against medical advice and 4 who had taken unsanctioned leave. Examination of the dimension scores of these subgroups revealed marked pattern differences which appeared to be consistent for the subjects within each subgroup. It appeared that the different ways in which these two subgroups terminated hospitalization might be related to basic personality differences. In table 9 are shown the group mean Q-sort scores of these subgroups along with those of a group of patients who received disciplinary discharges and a group of maximal hospital benefit patients.

Examination of table 9 yields several observations. As mentioned, the discharge against medical advice subgroup appears to differ considerably from the unsanctioned discharge subgroup, all 4 patients in the latter group obtaining

TABLE 9

MEAN SCORES OF DISCHARGE SUBGROUPS FOR DIMENSIONS A, C, AND D

Type of Discharge	N	Mean Stay	Dimension A			Dimension C		Dimension D		
			D	R	SA	P	N	OR	NR	UR
Against medical advice	6	203	115	133	112	199	161	124	126	110
Unsanctioned	4	237	128	112	120	222	138	118	119	123
Disciplinary	4	347	113	127	120	218	142	115	126	119
Maximal hospital benefit	10	374	119	135	106	215	145	116	129	115

TABLE 10

THE RELATIONSHIP BETWEEN WARD BEHAVIOR AND MEDICAL PROGRESS IN A SAMPLE OF TUBERCULOUS PATIENTS

	N	Medical Progress at 4 Months	
		Satisfactory	Unsatisfactory
		per cent	*per cent*
Good behavior group	12	67	33
Poor behavior group	9	67	33

high dependency, low regulation, and high self-assertion scores on the *A* dimension. (This arrangement of subscores within the *A* dimension appears only in one patient in any of the other subgroups: in one of the disciplinary discharges.) It was noted also that only in the disciplinary subgroup is the self-assertion score so high as to overbalance dependency within the *A* dimension. It may be noted, moreover, that the differences between the discharge against medical advice and the maximal hospital benefit groups are consistent with the original predictions made.

Although the final evaluation of these apparent differences must rest in experimental investigation, a tentative implication of these observations is that unsanctioned discharge does not appear to be a homogeneous behavior category, and that the specific forms which hospital leave-taking assumes are expressive of *different* patterns of psychologic needs, habits, and defenses. If this is so, psychologically meaningful studies will require investigation of the underlying dynamics of specific behavior forms, rather than of the unsanctioned discharge group or of the "voluntary" group taken as a supposed homogeneous whole.

An observation on an inter-behavior relationship: For many people in the field of tuberculosis, the importance of the bed-rest regimen is basic in the successful treatment of the disease. Others dispute the necessity of the rigid bed-rest schedule. Since independent and reliable measures of patients' behavior in the areas of ward behavior and medical progress were available, it was possible to examine the relationship between these two areas of behavior.

The measure of ward behavior used in this study, although not based solely on adherence to bed-rest schedules, does include this phase of ward life, and, in general, tests the patient's cooperation with the over-all regimen of the bed-rest

wards. Of the 28 patients in the ward behavior groups, 21 were subsequently suitable for rating in the medical progress area. In table 10 the relationship found between the two behavior forms is described; no systematic relationship is indicated.

Since the strictness of the therapeutic regimen is one of the most frustrating aspects of hospital life for the patient and is for many patients an important provocation for premature departure, the question of the medical necessity for extremely rigid rules is a practically important one, which apparently deserves more extensive objective study.

Summary

This study concerns an investigation of the relationship between selected personality dimensions and several measures of overt behavior among hospitalized tuberculosis patients. A structured Q-sort was constructed to measure the selected dimensions of personality: (*a*) the patient's characteristic way of relating himself to his environment in terms of dependency, regulation, and self-assertion; (*b*) whether the patient perceived the source of initiative in his hospital life situation as coming predominantly from himself or from the environment; (*c*) whether the patient perceived his environment as friendly and permissive or unfriendly and restrictive; and (*d*) the characteristic intensity of the patient's reactions to the various aspects of his hospital situation in terms of over-reaction, normal reaction, and under-reaction. Measures of these dimensions of personality were obtained at an early point in each patient's hospitalization. The areas of behavior studied were the patient's ward behavior, his rate of medical progress, and the way in which his treatment was terminated. Measures of these behavior areas were obtained at predetermined points of hospitalization. Specific predictions were made about the expected relationships between personality dimensions and behavior forms.

In the area of ward behavior, patients rated as displaying good ward behavior were found to be significantly more dependent and less self-assertive than patients rated as displaying poor ward behavior. The "good" patients also perceived their environment to be more friendly and permissive than did the "poor" patients. In the medical treatment area, it was found that patients who did not respond satisfactorily to medical treatment were significantly higher on over-reaction and lower on normal reaction than patients who made satisfactory medical progress. It was speculated that psychologic reactivity patterns are related to somatic functioning, with consequent effect on response to treatment. In the termination of treatment area, none of the predictions involving the four personality dimensions was confirmed. A posteriori examination of the data led to the hypothesis that the general category of irregular discharge is too heterogeneous in behavior to be explained in terms of one set of psychologic motivations, but that the several specific ways of terminating treatment might be meaningfully differentiated in terms of varying Q-sort personality patterns. Examination of the relationship between ward behavior and medical progress suggested that no relationship existed between these behavior forms. The question of the medical

necessity for a rigid hospital regimen in the treatment of tuberculosis was raised, and the need for research into the question was cited.

It appears that the manifest behavior of hospitalized tuberculous patients, both in its voluntary aspects (such as ward behavior) and in its involuntary aspects (such as physiologic response to medical treatment), is to a significant extent a function of personality factors. Such factors can apparently be identified, operationally defined, and reliably assessed. Some of these personality variables, assessed early in hospitalization, are related predictably to subsequent behavior patterns. It would seem that knowledge of the nature of these personality factors and an understanding of their dynamic relationships with behavior should facilitate the development of methods for constructive intervention in the psychodynamics of hospital life for the purpose of making the treatment process more effective. The general implication of the findings of this study supports the view that comprehensive management of tuberculosis must take the total person and not only his physical disease as the object of treatment.

Sumario

Algunas Relaciones entre la Personalidad y el Comportamiento en los Tuberculosos Hospitalizados

Versa este estudio sobre una investigación de la relación observada entre dimensiones escogidas de la personalidad y varias medidas del comportamiento público entre tuberculosos hospitalizados. Construyóse una especie de figura Q para medir las dimensiones de la personalidad escogidas: (*a*) la típica forma en que el enfermo se relacionaba con su ambiente en términos de dependencia, reglamentación y mantenimiento de sus derechos; (*b*) si el enfermo creía que la iniciativa en su vida hospitalaria partía predominantemente de sí propio o del ambiente; (*c*) si el enfermo concebía el ambiente como amistoso y tolerante o como hostil y restrictivo; y (*d*) la típica intensidad de las reacciones del enfermo a las varias fases de su situación hospitalaria en términos de hiperreacción, reacción normal y subrreacción. Estas mediciones de las dimensiones de la personalidad se obtuvieron en un punto temprano de la hospitalización de cada enfermo. Las zonas de comportamiento estudiadas fueron el comportamiento del enfermo en la sala, la velocidad de su progreso médico y la forma en que había terminado su tratamiento. Las mediciones de estas zonas de comportamiento se obtuvieron al llegar a ciertos puntos predeterminados de la hospitalización. Se formularon predicciones específicas acerca de las relaciones anticipadas entre las dimensiones de la personalidad y las formas de comportamiento.

En la esfera del comportamiento en la sala, se descubrió que los enfermos clasificados como mostrando buen comportamiento en las salas estaban decididamente más atenidos a otros y eran menos independientes que los clasificados como mostrando mal comportamiento. Los enfermos "buenos" también concebían su ambiente como más amistoso y tolerante que los "malos". En las esfera del tratamiento médico observóse que los enfermos que norespondían satisfactoriamente a dicho tratamiento ocupaban un puesto significativamente más alto en hiperreacción y más bajo en reacción normal que los que mostraban progreso médico satisfactorio. Se teorizó que los patrones de la reactividad psicológica se relacionan con el funcionamiento somático, con el consiguiente efecto sobre la reacción a la terapéutica. En la zona de la terminación del tratamiento, no se confirmó ninguna de las predicciones basadas en las cuatro dimensiones de la personalidad. El análisis *a posteriori* de los datos disponibles condujo a la hipótesis de que la categoría general de las altas irregulares es demasiado heterogéned en lo tocante a comportamiento para poder explicarlo en términos de una serie de motivos psicológicos, pero que los varios modos específicos de terminar el tratamiento podrían diferenciarse significativamente en términos de varios patrones de

personalidad estilo Q. Se suscitó el punto de la necesidad médica de mantener un rígido régimen hospitalario en el tratamiento de la tuberculosis y se citó la necesidad de investigar la cuestión.

Parece que el comportamiento manifiesto de los tuberculosos hospitalizados, tanto en sus fases voluntarias (p. ej., el comportamiento en las salas) como involuntarias (p. ej., la respuesta psicológica al tratamiento médico), es, en grado importante, una función de ciertos factores de la personalidad. Esos factores pueden aparentemente identificarse, definirse en su acción y justipreciarse debidamente. Algunas de esas variables de la personalidad, justipreciadas en los principios de la hospitalización, se relacionan en forma predecible con los patrones del comportamiento subsiguiente. Parece que el conocimiento de la naturaleza de estos factores de la personalidad y la comprensión de sus relaciones dinámicas con el comportamiento deben facilitar la elaboración de métodos para la intervención constructiva en la psicodinámica de la vida hospitalaria con el objeto de impartir mayor eficacia al proceso terapéutico. La tendencia general de los hallazgos de este estudio apoya la opinión de que la asistencia comprensiva de la tuberculosis debe tomar por mira terapéutica a la persona en conjunto y no puramente su afección física.

Resume

De certains rapports entre la personnalité et le comportement chez les malades tuberculeux hospitalisés

Cette étude concerne une investigation de la relation entre certains traits sélectionnés de la personnalité et diverses mesures du comportement apparent parmi des malades tuberculeux hospitalisés. Une méthode "structured Q-sort" a été établie pour mesurer les traits sélectionnés de la personnalité: a) L'attitude caractéristique du patient dans ses relations avec le milieu, en termes de dépendance, d'attitude envers les règles et d'indépendance; b) Si le malade considère que la source de toute activité dans sa vie hospitalière dérive surtout de sa propre volonté ou est déterminée par le milieu; c) si le malade conçoit son milieu comme amical et bienveillant ou inamical et hostile, ainsi que l'intensité spécifique des réactions du malade aux divers aspects de sa situation à l'hôpital en termes d'hyperréactivité, de réactivité normale et d'hyporéactivité. Les déterminations de ces traits de la personnalité furent obtenues des le début de l'hospitalisation chez chaque malade. Les aspects du comportement étudiés étaient l'attitude du patient dans la salle commune, la vitesse de ses progrès du point de vue médical et la manière dont son traitement se terminait. Les déterminations de ces aspects du comportement furent effectuées en des temps prédéterminés de la période d'hospitalisation. Des prévisions spécifiques étaient établies quant aux rapports ultérieurs entre les traits de la personnalité et les modalités du comportement.

A l'égard du comportement dans la salle commune, les patients évalués comme faisant preuve d'un bon comportement furent trouvés notablement plus dépendants et moins agressifs que les malades évalués comme faisant preuve d'un mauvais comportement. Les "bons" patients considéraient que leur milieu était plus amical et plus bienveillant que ne le concevaient les "mauvais" patients. A l'égard du traitement médical, il a été découvert que les malades qui ne répondaient pas de façon satisfaisante au traitement médical montraient dans les déterminations, une hyperréactivité notablement plus élevée et une réaction normale beaucoup plus basse, que les patients qui accomplissaient des progrès satisfaisants au point de vue médical. On prévoyait que les modalités de la réactivité psychologique étaient en relation avec l'état fonctionnel somatique, d'ou l'effet sur la réaction au traitement. A l'égard de la manière dont le traitement se terminait, aucune des prévisions condoncernant les traits de la personnalité ne furent confirmées. Un examen ultérieur des connées a conduit à la conception que dans la catégorie générale des départs irréguliers de l'hôpital le point de vue comportement est trop hétérogène pour être expliqué en termes de motivations psychologiques s'appliquant à un seul groupe, mais que les différentes façons spécifiques de finir le traitement pourraient avec raison être différenciées en termes de divers types de personnalités "Q-sort."

L'examen des relations entre le comportement dans la salle commune et les progrès, du point de vue médical suggérait qu'il n'existait aucun rapport entre ces formes de comportement. Le problème de la nècessité médicale d'un régime hospitalier rigide dans le traitement de la tuberculose a été posé, et la nécessité d'étudier ce problème ont été mentionnés.

Il semble que le comportement des malades tuberculeux hospitalisés, à la fois sous ses aspects volontaires (tel que le comportement dans la salle) et sous son aspect involontaire (tel que la réponse physiologique au traitement médical), est à un degré significatif une fonction des facteurs de la personnalité. Ces facteurs paraissent pouvoir être identifiés, définis mathématiquement et évalués de façon sûre. Certaines de ces variables de la personnalité évaluées dès le début de l'hospitalisation pourraient être rapportées par prévision aux modalités subséquentes du comportement. Il semblerait que la connaissance de la nature de ces facteurs de la personnalité et la compréhension de leurs rapports dynamiques avec le comportement devraient faciliter le développement de méthodes permettant une intervention constructive dans la psychodynamie de la vie à l'hôpital dans le but d'accroître l'efficacité des procédés thérapeutiques. L'implication générale des données de cette étude corrobore la conception qu'un traitement complet compréhensif de la tuberculose doit tenir compte de l'individu dans son ensemble et non seulement de sa maladie physique comme objet du traitement.

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PROTEINS AND MUCOPROTEINS IN PLEURAL EFFUSIONS[1]

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INTRODUCTION

The proteins contained in pleural effusions have been the subject of several investigations (1–8). There is general agreement that the total proteins are higher in pleural effusions, due to inflammatory and neoplastic processes, than those due to congestive failure, hepatic cirrhosis, or nephrotic syndrome.

Regardless of the underlying disease, pleural fluid contains all of the major protein fractions of the blood serum and probably originates from it. Irrespective of the total amount of proteins, however, the albumin fraction is relatively higher in the pleural fluid than in the serum of the respective patient, whereas the globulins are relatively lower (2, 4–6). Fibrinogen concentration is either low or absent in pleural effusions, for it may be precipitated on the pleura as a thick fibrin deposit (1, 2). Deviations of the percentage of alpha and gamma globulins, increases as well as decreases, have been described as characteristic for some conditions (5, 8). These data, however, are not sufficiently distinctive to provide diagnostic differentiation. Occasionally one encounters a diagnostic problem concerning the cause of a pleural effusion, and the differentiation between neoplastic disease and tuberculosis can become particularly difficult.

It was considered worth while to measure the total proteins and other constituents of pleural fluids in order to learn whether these measurements might contribute to the diagnostic differentiation of pleural effusions. Determinations were made of the protein fractions obtained electrophoretically and the total mucoproteins in pleural fluids, the specific gravity, and the cellular elements contained in the fluids. An increase in the mucoproteins of the *serum* has been found in 90 per cent of patients with visceral carcinoma or lymphomas, but only in 75 per cent of patients with subacute or chronic inflammatory disease (9).

MATERIALS AND METHODS

Fluid aspirated from pleural effusions of 52 patients was collected in sterile 50 ml. test tubes, containing 0.1 mg. of lithium oxalate. The patients were divided into five groups according to the diagnosis of the basic lesion responsible for the pleural effusion (carcinoma, tuberculosis, lymphoblastoma, pneumonia, transudates). The diagnosis of carcinoma and lymphoblastoma was made on the evidence of surgical biopsy or autopsy. Eight of the 12 tuberculous pleural effusions were proved by culture; in the remaining 4 the diagnosis was made on clinical and roentgenographic evidence. Three of the 6 postpneumonic effusions were confirmed by bacteriologic evidence and the other 3, by surgical intervention or autopsy.

The pleural fluid was either processed immediately or kept in a deep-freeze chest prior to the quantitative determination of proteins and mucoproteins (10, 11, 13). The total amount

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TABLE 1

PLEURAL EFFUSIONS DUE TO CARCINOMA

Patient Number	Pathologic Lesion	Specific Gravity	Erythrocytes	Leukocytes	Lymphocytes	Total Protein	Albumin	Globulins Per Cent					Mucoproteins
								α_1	α_2	β	ϕ	γ	
			per cu. mm.	*per cu. mm.*	*per cent*	*gm. per 100 ml.*	*per cent*						*in mg. per 100 ml.*
1	Bronchogenic carcinoma	1.017	14,100	933	100	3.3	38.3	7.5	11.2	12.4	9.5	21.1	102.1
2	Adenocarcinoma, breast	1.020	10,300	280	57	4.0	52.1	5.4	7.6	8.5	6.3	20.1	86.3
3	Adenocarcinoma, breast					3.0	66.0	5.5	4.6	12.4	4.5	7.0	57.0
4	Bronchogenic carcinoma	1.026		1,350	80	4.2	45.6	6.4	8.4	8.9	17.0	13.6	46.3
5	Hypernephroma, metastatic					1.6	49.7	6.2	8.0	13.3	6.8	16.0	52.5
6	Adenocarcinoma		300	600		4.0	47.7	5.4	8.6	13.7	13.9	10.7	80.3
7	Carcinoma (lung) undifferentiated	1.020				4.4	60.6	2.2	6.1	10.6	3.9	16.6	62.5
8	Bronchogenic carcinoma	1.016	73,800	30,800	87	3.3	57.7	4.1	6.8	12.1	1.4	17.9	46.7
9	Bronchogenic carcinoma	1.022	3,400	0		2.3	43.1	4.9	6.9	9.8	0.5	34.8	64.3
10	Small cell carcinoma (lung)	1.027				3.3	32.9	6.8	8.2	10.5	2.0	39.6	69.7
Mean						3.3	49.4	5.4	7.6	11.2	6.6	19.7	66.8

of protein was measured by the Kingsley method (12). The major protein fractions were determined by filter paper electrophoresis. The hanging strip method was used, employing Whatman No. 3 filter paper, a direct current of 6 milliamperes for a period of ten hours and a veronal buffer of pH 8.6, and the ionic strength of 0.05 M. The strips were dried in an oven at 100°C. and then stained with tetrabromophenolsulphonphthalein (bromphenol-blue). The electrophoretic curves were obtained from the stained strips by means of densitometry and were measured quantitatively by planimetry.

The data on specific gravity and cell counts were obtained, wherever available, from the clinical records, and are incomplete.

After electrophoresis the paper was stained not only for proteins, but also with periodic acid-Schiff stain (14) in order to obtain information on the relative distribution of carbohydrates in the protein fractions. The method was not accurately reproducible in this laboratory; hence, these data are not included in this report.

Results

The data from 10 pleural effusions due to carcinoma are contained in table 1. The specific gravity ranged from 1.016 to 1.027; the eyrthrocyte counts, from 300 to 73,800; the leukocytes, from 0 to 30,800 per cu. mm. The major part of the leukocytes consisted of mononuclear cells. The total proteins ranged from 1.6 to 4.4 gm. per 100 ml., with a mean of 3.3 gm. The mucoproteins ranged from 46.3 to 102.1 mg. per 100 ml., with a mean of 66.8 mg.

The determinations made in 12 tuberculous effusions are presented in table 2. The specific gravity ranged from 1.015 to 1.027, the erythrocytes from 0 to 30,800, the leukocytes from 520 to 8,750 per cu. mm. Polymorphonuclear cells predominated in two of these fluids, mononuclear cells in the remainder. The

TABLE 2

Pleural Effusions Due to Tuberculosis

Patient Number	Pathologic Lesion	Specific Gravity	Erythrocytes	Leukocytes	Lymphocytes	Total Protein	Albumin	Globulins Per Cent					Mucoproteins
								γ_1	γ_2	β	ϕ	γ	
			per cu. mm.	*per cu. mm*	*per cent*	*gm. per 100 ml.*	*per cent*						*in mg. per 100 per ml.*
11	Tuberculosis		4,550	2,450	100	5.3	49.7	3.7	13.8	9.1	4.9	18.8	131.9
12	Tuberculosis					4.1	63.8	1.0	3.0	12.1	8.1	12.0	51.0
13	Tuberculosis	1.030	1,050	520	90	5.5	40.9	2.6	5.5	7.8	23.2	20.0	39.3
14	Tuberculosis					4.5	40.8	2.9	6.8	9.1	16.9	23.5	49.4
15	Tuberculosis					3.2	65.2	2.6	8.2	8.5	0	15.5	39.8
16	Tuberculosis					4.3	51.6	4.5	8.5	13.8	11.6	10.0	52.2
17	Tuberculosis	1.015	0	3,600	85	5.1	57.4	4.1	5.6	15.5	8.8	8.6	24.9
18	Tuberculosis	1.022	19,000	3,000	100	5.3	34.7	3.6	5.4	5.8	0.7	49.8	73.0
19	Tuberculosis	1.021	11,000	520	31	6.0	46.0	4.0	8.3	6.2	1.9	33.6	112.3
20	Tuberculosis		15,650	8,750	53	5.7	43.8	5.2	7.7	15.1	1.2	27.0	101.9
21	Tuberculosis and hepatic cirrhosis	1.020	30,800	2,250	13	3.1	30.0	4.1	8.5	8.7	7.4	41.3	41.5
22	Tuberculosis	1.027	15,750	3,750	60	6.4	38.5	3.9	13.0	10.3	0	34.3	127.2
Mean						4.9	46.9	3.5	7.6	10.2	7.0	24.5	70.4

TABLE 3

PLEURAL EFFUSIONS DUE TO LEUKEMIA AND LYMPHOBLASTOMAS

Patient Number	Pathologic Lesion	Specific Gravity	Erythrocytes	Leukocytes	Lymphocytes	Total Protein	Albumin	Globulins Per Cent					Mucoproteins
								α_1	α_2	β	ϕ	γ	
			per cu. mm.	*per cu. mm.*	*per cent*	*gm. per 100 ml.*	*per cent*						*in mg. per 100 ml.*
23	Hodgkins disease	1.018	0	2,580	50	4.3	50.3	5.2	12.6	9.6	0	22.3	93.6
24	Chronic lymphocytic leukemia					3.3	55.5	7.0	6.2	11.3	6.0	14.0	33.2
25	Reticulum cell sarcoma					3.3	47.7	4.8	10.9	11.8	14.8	10.0	60.3
26	Chronic lymphocytic leukemia		35,000	1,650	88	3.6	55.0	5.2	6.2	3.8	1.4	28.4	51.6
27	Hodgkins disease		91,000	2,550	69	5.0	49.3	3.6	5.3	8.7	0	33.1	174.1
Mean						3.9	51.6	5.2	8.2	9.0	4.4	21.6	82.6

TABLE 4

PLEURAL EFFUSIONS DUE TO INFECTION OTHER THAN TUBERCULOSIS

Patient Number	Pathologic Lesion	Specific avity	Erythrocytes	Leukocytes	Lymphocytes	Total Protein	Albumin	Globulins Per Cent					Mucoproteins
								α_1	α_2	β	ϕ	γ	
			per cu. mm.	*per cu. mm.*	*per cent*	*gm. per 100 ml.*	*per cent*						*in mg. per 100 ml.*
28	Pneumonia in congestive failure	1.015		32		6.0	47.8	2.3	6.0	6.9	7.4	29.6	76.7
29	Pneumococcal pneumonia					3.6	42.5	4.5	8.4	19.4	9.1	16.1	28.6
30	Staphylococcal pneumonia					1.8	39.3	7.7	10.6	10.1	0	32.3	74.5
31	Pneumococcal pneumonia					4.4	49.6	8.6	10.1	9.4	0	22.3	98.4
32	Septic pulmonary emboli	1.020	11,500	1,400	76	5.1	50.3	7.5	9.6	7.5	0	25.1	136.2
33	Pneumococcal pneumonia	1.023	16,100	2,150	44	4.1	45.2	5.2	8.4	6.9	0	34.3	38.3
Mean						4.2	45.8	5.9	8.9	10.0	2.8	26.6	75.5

total proteins ranged from 3.1 to 6.4 gm. per 100 ml., with a mean of 4.9 gm. The mucoproteins ranged from 24.9 to 127.2 mg. per 100 ml., with a mean of 70.4 mg.

Data from 5 pleural effusions associated with lymphoblastomas are contained in table 3. The specific gravity of only one specimen had been obtained. The erythrocytes ranged from 0 to 91,000, the leukocytes from 1,650 to 2,580 per cu. mm. There was no specimen in which the polymorphonuclear cells predominated. The total proteins ranged from 3.3 to 5.0 gm. per 100 ml. with a mean of 3.9 gm. The mucoproteins ranged from 33.2 mg. to 174.1 mg. per 100 ml., with a mean of 82.6 mg.

The results with 6 postpneumonic effusions are shown in table 4. The specific

TABLE 5

PLEURAL EFFUSIONS ASSOCIATED WITH WATER AND SALT RETENTION

Patient Number	Pathologic Lesion	Specific Gravity	Erythrocytes	Leukocytes	Lymphocytes	Total Protein	Albumin	Globulins Per Cent					Mucoproteins
								α_1	α_2	β	ϕ	γ	
			per cu. mm.	*per cu. mm.*	*per cent*	*gm. per 100 ml.*	*per cent*						*in mg. per 100 ml.*
34	Congestive failure		0	445	100	2.6	58.4	3.5	4.0	9.0	0	25.1	33.1
35	Hepatic cirrhosis					1.0	43.0	6.0	0	6.0	11.3	33.7	15.8
36	Intercapillary glomerulosclerosis	1.016				1.2	45.2	7.6	9.2	12.4	12.7	12.9	48.3
37	Intercapillary glomerulosclerosis	1.019		80	80	2.3	50.4	4.6	7.8	10.8	9.4	17.0	62.4
38	Congestive failure	1.010		76	78	1.9	53.4	5.2	3.9	8.7	4.7	24.1	13.0
39	Congestive failure	1.011		95	90	2.3	71.5	4.2	3.0	11.5	3.3	6.5	40.4
40	Hepatic cirrhosis		4,450	200	100	0.7	36.9	10.7	7.0	13.1	0.0	32.3	19.9
41	Congestive failure		3,000	350		1.6	45.4	5.8	7.9	9.9	0.7	30.3	57.6
42	Polyarteritis nodosa		3,950	3,400	26	2.1	39.1	7.7	11.5	9.6	4.4	27.7	113.9
43	Congestive failure		2,250	2,100	93	3.3	51.0	6.7	4.6	10.1	1.0	26.6	40.4
44	Hepatic cirrhosis	1.015				1.5	37.8	7.7	7.3	11.4	0	35.8	12.2
45	Hepatic cirrhosis	1.026	1,000	350	100	0.7	47.8	3.7	10.5	16.7	2.5	18.8	74.5
46	Porphyria hepatica	1.013	600	40	41	1.7	56.5	5.2	5.5	12.2	0	20.6	13.7
47	Congestive failure	1.008		58	94	1.9	61.2	2.8	2.8	5.1	0	28.1	37.1
48	Congestive failure	1.010	Full field	1,480	95	1.9	49.8	6.4	7.5	7.5	2.7	26.1	64.9
49	Hepatic cirrhosis					1.5	29.6	3.9	6.9	18.1	8.0	33.5	15.3
50	Congestive failure	1.015	6,750	200	50	2.6	56.6	2.0	6.1	10.1	2.0	23.2	28.9
51	Congestive failure		2,650	1,150	75	1.0	39.6	6.2	8.1	11.6	0	34.5	85.6
52	Hepatic cirrhosis		1,000	350	100	0.7	37.9	4.0	5.1	9.2	1.5	42.3	19.7
Mean						1.7	48.0	5.5	6.2	10.6	3.4	26.3	42.0

gravity of one specimen was 1.015, in two others it was 1.020 and 1.023, respectively. Leukocyte counts were obtained in three specimens and ranged from 32 to 2,150 per cu. mm. The total proteins ranged from 1.8 to 6.0 gm. per 100 ml., with a mean of 4.2 gm.; the mucoproteins from 28.6 to 136.2 mg. per 100 ml., with a mean of 75.5 mg.

In table 5 are contained data from 19 transudates due to congestive failure, hepatic cirrhosis, nephrotic syndrome, or other conditions giving rise to fluid retention. The specific gravity of these pleural fluids ranged from 1.010 to 1.026. From 0 to 6,750 erythrocytes per cu. mm. were counted, but one effusion (No. 48) was grossly bloody and was noted to show "a full field of cells." The leukocytes ranged from 40 to 3,400 per cu. mm. and, in 11 of 14 specimens evaluated cytologically, showed a predominance of mononuclear leukocytes. The total proteins ranged from 0.7 gm. to 3.3 gm. per 100 ml., with a mean of 1.7 gm. The mucoproteins ranged from 12.2 mg. to 113.9 mg. per 100 ml., with a mean of 42.0 mg.

The 12 tuberculous effusions (table 2) thus showed the highest average total proteins at 4.9 gm. per 100 ml., closely followed by the postpneumonic effusions

with a mean of 4.2 gm. (table 4). The effusions due to lymphoblastoma (table 3) and carcinoma (table 1) followed with 3.9 gm. and 3.3 gm. per 100 ml., respectively. The mean total proteins of the 19 transudates (table 5) was 1.7 gm. per 100 ml.

The total mucoproteins in effusions due to carcinoma, lymphoblastoma, or infection were higher than those in the transudates (table 5), but there was no close correlation with the total amount of proteins.

Discussion

Simultaneous electrophoretic studies of the serum and pleural effusion of some of the patients bore out previous statements that the electrophoretic pattern of the pleural fluid in a given patient is essentially an image of the plasma pattern with the exception of fibrinogen, which is decreased or absent. Two patients, not included in the present study, one with multiple myeloma and the other with congenital hypogammaglobulinemia ("agammaglobulinemia"), had abnormal protein patterns in their pleural effusions as well as in their serum. For these reasons the present writers soon ceased to determine protein fractions in both plasma and pleural fluids, particularly since it was the purpose of this study to examine the pleural fluids for their immediate diagnostic value.

It is apparent from tables 1 to 5 that the specific gravity of the pleural fluids due to carcinoma, tuberculosis, lymphoblastoma, and postpneumonic effusion is higher than that of the transudates (table 5). However, there is some overlap beyond the customary value of 1.016, which is presumed to separate exudates from transudates. This confirms recent data by Leuallen and Carr (7). Keller suggested that a broadening of the dividing zone might be helpful, but admitted that even this failed to separate all transudates from exudates (6).

The distribution and relative number of cellular elements are not particularly helpful in the differential diagnosis of a given pleural effusion. Thus, it may be seen that the cytologic picture of a tuberculous effusion is not always dominated by lymphocytes, and the presence of a bloody fluid does not militate against a tuberculous origin. This confirms the data of Leuallen and Carr (7), and also is in agreement with the observations of Jacobaeus, who noted ulcerative, bleeding lesions along with miliary nodules and tumor-like infiltrates in numerous thoracoscopic examinations of pleural tuberculosis (15).

The values for the total proteins in pleural fluids showed a fair separation between transudates (table 5) and the exudates due to carcinoma, lymphoma, postpneumonic effusion, or tuberculosis (tables 1 to 4). If the arbitrary line of separation is placed at 3 gm. per 100 ml., one may notice that none of the tuberculous effusions or those due to lymphoblastoma contained total proteins below this dividing line. One effusion due to bronchogenic carcinoma, one due to malignant nephroma, and one postpneumonic effusion, however, had total proteins below 3 gm. per 100 ml. These exceptions amount to 3 of a total of 33 exudates. The 19 transudates presented in table 5 showed only one value higher than 3 gm. per 100 ml. Previous investigations (4, 7) have also shown the values for total proteins in pleural fluids to be most useful in the differentiation of transudates from exudates, although there also was some degree of overlapping.

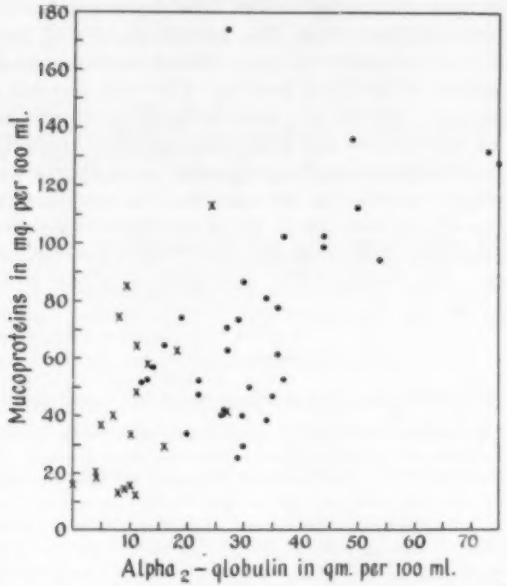

FIG. 1: The relation of mucoproteins to alpha$_2$ globulins in 52 pleural effusions is demonstrated graphically. The pleural effusions due to transudates are marked (x) whereas the effusions due to carcinoma, tuberculosis, lymphoblastoma, or postpneumonic effusion are indicated by dots. The correlation of the transudates is poor, whereas the other effusions show a consistent pattern.

The quantitative determination of the protein fractions as well as of the mucoproteins in the pleural fluids failed to add essential information of diagnostic usefulness. The average amount of mucoproteins in the exudative effusions presented in tables 1 to 4 was higher than that of the transudates (table 5), but the pattern was not consistent. The values did not parallel those of the total proteins. There was an excellent correlation between the quantity of mucoproteins and alpha$_2$ globulins in the pleural fluids due to neoplasms, lymphoblastomas, and infections (tables 1–4), with the highly significant correlation coefficient of 0.591. However, when the transudates in table 5 were added to the total, the correlation coefficient decreased to a value of 0.33 (figure 1).

These data imply that the mucoproteins in pleural effusions due to neoplasms, lymphoblastomas, or infections are associated preponderantly with the alpha$_2$ globulin fraction, whereas this seems to be true to a lesser extent in the effusions due to transudates.

SUMMARY

All of the major serum protein fractions are contained in pleural effusions in a distribution similar to that in the serum. Fibrinogen, however, may be decreased or absent in the aspirated fluid. Pleural effusions due to frankly inflammatory

processes contain more total proteins than those due to neoplasms, but there is a considerable overlap in the values. The specific gravity of the pleural fluids examined in this study separated transudates from exudates less effectively than did the concentration of the total proteins. The total proteins of transudates usually are below 3 gm. per 100 ml., whereas those in exudates are greater.

The number of erythrocytes and leukocytes as well as the differential counts of the leukocytes in these pleural fluids appeared to be of little diagnostic value. The quantitative determination of mucoproteins in these pleural effusions failed to add information of diagnostic value. There was a good correlation between the amounts of mucoproteins and alpha$_2$-globulins in the effusions due to neoplasms, lymphoblastomas, and infections. This correlation held only to a less extent in the transudates.

Sumario

Las Proteínas y las Mucoproteínas en los Derrames Pleurales

Todas las grandes fracciones de la seroproteína aparecen en los derrames pleurales en una proporción semejante a la alcanzada en el suero. No obstante, el fibrinógeno puede disminuir o faltar en el líquido aspirado. Los derrames pleurales debidos a procesos francamente inflamatorios contienen más proteínas totales que los debidos a neoplasias. Sin embargo, existe considerable sobreposición en los valores. Los valores de la densidad de los líquidos pleurales analizados en este estudio separaron los trasudados de los exudados menos positivamente que la concentración de las proteínas totales. Las proteínas totales de los trasudados suelen quedar por debajo de 3 gm. por 100 ml., mientras que las de los exudados son más altas.

El número de eritrocitos y de leucocitos así como las fórmulas diferenciales de los leucocitos en esos líquidos pleurales parecieron poseer poco valor diagnóstico. La determinación cuantitativa de las mucoproteínas en estos derrames pleurales no añadió la menor información de valor diagnóstico. Hubo buena correlación entre las cantidades de mucoproteínas y de alfa$_2$-globulinas en los derrames debidos a neoplasias, linfoblastomas e infecciones. Esta correlatión no se mantuvo más que en menor grado en los trasudados.

Resume

Les protéines et les mucoprotéines dans les épanchements pleuraux

Toutes les fractions protéiques majeures du sérum sont contenues dans les épanchements pleuraux et leur répartition est semblable à celle existant dans le sérum. Toutefois, le fibrinogène peut être réduit ou absent dans le liquide de ponction. Les épanchements pleuraux dûs aux processus réellement inflammatoires contiennent une quantité de protéines totales plus importante que ceux dûs aux néoplasmes. Il y a toutefois de grandes similarités dans les valeurs. Les valeurs du poids spécifique des liquides pleuraux examinés dans cette étude n'ont pas permis de différencier les transsudats des exsudats aussi efficacement que ne le permettait la concentration des protéines totales. Les protéines totales des transsudats sont habituellement inférieures à 3 g/100 tandis que leur valeur est plus élevée dans les exsudats.

Le nombre des érythrocytes et des leucocytes ainsi que la numération différentielle des leucocytes dans ces liquides pleuraux paraissaient avoir une faible valeur diagnostique. La détermination quantitative des muco-protéines dans ces épanchements pleuraux n'a pas ajouté d'informations de valeur diagnostique. La corrélation entre les quantités de muco-protéines et les alpha$_2$-globulines dans les épanchements dûs aux néoplasies, aux lymphoblastomes et aux infections était bonne. Cette corrélation était moins valable pour les transsudats.

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PRELIMINARY EXPERIMENTS ON PASSIVE TRANSFER OF PROTECTIVE HUMORAL ANTIBODIES IN TUBERCULOSIS[1, 2]

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INTRODUCTION

Ever since Robert Koch discovered *M. tuberculosis*, the riddle of immunity to tuberculous infection has been explored. Nevertheless, it remains essentially unsolved. Many facets of host immunity have been investigated. The present studies are concerned with immunity associated with the production of humoral antibodies.

The prevalent view of immunity in tuberculosis was expressed by Zinsser, Enders, and Fothergill (1): "It is . . . generally accepted that the known antibodies which react either with the bacilli or products derived from them . . . are unable to enhance the resistance of the animal." However, some passive transfer experiments conducted more than thirty years ago would appear to refute this statement. Römer (2) infected four tuberculin-negative sheep with equal doses of tubercle bacilli. He treated two of them with the serum of immunized sheep, one injection of 10 ml. each week for five weeks. The control sheep died six weeks after infection with extreme, generalized tuberculosis. One of the immunized sheep was killed at the end of ten months while in good health; only a few encapsulated lesions in the lungs and peribronchial lymph nodes were seen. The other lived one and a half years and showed no trace of tuberculosis at autopsy. Neufeld and Lange (3) repeated Römer's experiment with larger numbers of sheep and larger amounts of immune serum. They concluded that the increased survival time of the treated animals indicated the presence of a protective factor in the serum. Mayer and Hurley (4), in another experiment, transfused one sheep with large amounts of blood from an immunized sheep. They found that the animal was highly protected against tuberculous infection, compared with sheep which received transfusions of normal sheep's blood.

The purpose of the present preliminary experiments was to determine whether protective antibodies could be demonstrated in human serum. Specifically, the experiments were designed to determine whether various globulin fractions of human serum from tuberculous patients could inhibit the growth of tubercle bacilli *in vitro* and *in vivo*.

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[2] This study was supported, in part, by the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association, made possible by a special bequest from the estate of Sophie Goman; by research grant 1-61-460 from the Institute of Allergy and Infectious Diseases, National Institutes of Health, Public Health Service.

METHOD

Blood was obtained from tuberculous patients who had had their disease for six months or longer and were doing well. After the blood clotted, the serum was removed and fractionated by the cold ethanol method of Cohn and his associates (5).

In vitro experiments: In order to test the inhibition of growth of tubercle bacilli *in vitro*, various test substances were added to tubes containing 0.1 ml. of a seven-day culture of the H37Rv strain of tubercle bacilli (density slightly less than No. 1 MacFarland barium sulfate nephelometer) in 4.5 ml. of Dubos Tween®-albumin-dextrose medium (6). For each experiment, 0.5 ml. of the test solution was added to tube No. 1. Serial dilutions of the test solutions were made in albumin-dextrose solution so that tube No. 2 contained 0.25 ml. of test substance and 0.25 ml. of albumin-dextrose solution, and tube No. 3 contained 0.125 ml. of test substance and 0.375 ml. of albumin-dextrose, and so forth (table 1).

The test substances consisted of: tuberculous serum; 10 per cent solution of fractions II-III of tuberculous serum (gamma and beta globulins); 10 per cent solution of fraction IV of tuberculous serum (alpha and beta globulins); immune gamma globulin (commercial); tuberculin-negative human serum; and 10 per cent bovine albumin. Control tubes prepared for each experiment contained medium and culture as above. To this, 0.5 ml. of albumin-dextrose solution was added in lieu of test solutions. Readings were made after seven and fourteen days of incubation at 37°C.

In vivo survival time experiments: (*1*) Sixty-one male Webster Swiss albino mice, weighing 18 to 20 gm., divided into groups as shown in table 2, were treated with daily injections of 0.5 ml. of human serum, or fractions thereof. The serum or fraction was administered in one dose before and six doses after intravenous infection with 0.2 ml. of a 10^4 dilution of a seven-day culture of the Vallée (Rockefeller Institute) strain of tubercle bacilli.

(*2*) One hundred and five male Webster Swiss albino mice, weighing 18 to 20 gm., divided into groups as shown in table 3, were treated with daily injections of 0.5 ml. of human serum or fractions thereof. Four doses were given before challenge and four doses after challenge with the Vallée strain of tubercle bacilli, as in (*1*).[2]

(*3*). Ninety-nine male Webster Swiss albino mice, weighing 18 to 20 gm., divided into groups as shown in table 4, were treated with four daily injections of 0.5 ml. of serum or fractions thereof before infection with Vallée strain of tubercle bacilli, as in (*1*) and (*2*). No serum or fractions were given after the challenge.

Since the tuberculous patients from whom serum was obtained had received antituberculous therapy, the fractions were tested for the presence of streptomycin, para aminosalicylic acid (PAS), and isoniazid. None was found. Histologic sections of the lungs of all animals are being examined for the presence of tuberculosis and acid-fast organisms.

RESULTS

In Vitro

The tests *in vitro* showed inhibition of growth of H37Rv with high concentrations of fractions II-III of tuberculous serum; of fraction IV of tuberculous serum; of unfractionated tuberculous serum; and of immune gamma globulin. The greatest inhibition occurred with fractions II-III of tuberculous serum. No

[2] Four additional groups were excluded from the table because laboratory accidents which occurred in these groups made it impossible to evaluate the results. The excluded groups had been given the following test substances: 10 per cent solution of fractions II-III of tuberculin-negative human serum; 10 per cent solution of fraction IV of tuberculin-negative human serum; 10 per cent solution of fractions II-III of tuberculin-positive human serum from healthy donors; 10 per cent solution of fractions II-III of tuberculous human serum. It is planned to repeat these studies in subsequent experiments.

TABLE 1

IN VITRO TEST FOR INHIBITION OF H37Rv BY VARIOUS PROTEIN FRACTIONS OF HUMAN SERUM

Test Substances	Time	Tubes—Concentration of Protein						Control
		1 0.5 ml.	2 0.25 ml.	3 0.125 ml.	4 0.063 ml.	5 0.016 ml.	6 0.008 ml.	
	weeks							
Tuberculous serum	1	+	+	C	C	++++	++++	++++
	2	+	+	C	C	++++	++++	++++
Fractions II and III of tuberculous serum —10%	1	−	−	−	−	−	−	++++
	2	−	−	++++	++++	++++	++++	++++
Fraction IV of tuberculous serum—10%	1	−	−	++++	++++	++++	++++	++++
	2	+	+	++++	++++	++++	++++	++++
Immune gamma globulin (commercial)	1	+	+	++++	++++	++++	++++	++++
	2	+	+	++++	++++	++++	++++	++++
Tuberculin - negative serum	1	++++	++++	++++	++++	++++	++++	++++
	2	++++	++++	++++	++++	++++	++++	++++
Albumin—10%	1	++++	++++	++++	++++	++++	++++	++++
	2	++++	++++	++++	++++	++++	++++	++++

− = No growth; + = Slight atypical growth; C = Contaminated; ++++ = Typical growth.

inhibition of growth was seen with tuberculin-negative serum and bovine albumin (table 1).

In Vivo Experiments

Median and mean survival times are given in the tables. The former is considered by the statisticians consulted to be the best measure of survival time in this type of experiment.

In *Experiment 1*, the group of animals which received immune gamma globulin had the longest survival time, according to both median and mean survival time. Then, in order, came the group which received fraction IV of tuberculous serum, fractions II-III of tuberculous serum, unfractionated tuberculous serum and, last, the controls (table 2). It should be noted that immune gamma globulin is a 16.5 per cent solution, and unfractionated serum contains about 2.5 per cent of globulin.

In *Experiment 2*, the best survival time occurred in the group which received fraction IV of human tuberculous serum. Then, in order, came the group which received fractions II-III of human tuberculous serum (given before challenge only), fraction IV of nontuberculous tuberculin-positive serum, immune gamma globulin, and the controls (table 3).

In *Experiment 3*, the best survival time occurred in the group which received fraction IV of human tuberculous serum. The second best group was the one given II-III of tuberculous serum. Then, in order, came the control group and the group which received unfractionated tuberculous serum. Last came the group

TABLE 2

SURVIVAL TIME OF SWISS ALBINO MICE* INFECTED WITH VALLÉE STRAIN† OF TUBERCLE BACILLI AND TREATED WITH PROTEIN FRACTIONS OF HUMAN SERUM

Group	Number	50 Per Cent Survival Time	Mean Survival Time
		days	*days*
A—saline	14	33.0	33.9
B—tuberculous serum	11	43.0	44.6
C—tuberculous serum fractions II–III—10%	10	44.0	44.7
D—tuberculous serum fraction IV—10%	9	47.5	44.3
E—gamma globulin (commercial)	17	53.5	48.9

* From Carworth Farms.

† Rockefeller strain.

Animals received one intraperitoneal injection of the test substance before infection and six injections of the test substance following infection, at daily intervals.

TABLE 3

SURVIVAL TIME OF SWISS ALBINO MICE INFECTED WITH VALLÉE STRAIN OF TUBERCLE BACILLI AND TREATED WITH PROTEIN FRACTIONS OF HUMAN SERUM

Group	Number	50 Per Cent Survival Time	Mean Survival Time
		days	*days*
A—saline	20	39.5	41.3
B—tuberculin positive serum fraction IV—10%	23	44.0	45.2
C—tuberculous serum fractions II–III—10%	16	50.5	53.1
D—tuberculous serum fraction IV—10%	24	58.5	57.1
E—gamma globulin (commercial)	22	47.0	44.1

Animals in groups *A*, *B*, *D*, and *E* received four daily intraperitoneal injections of the test substance before infection and four daily intraperitoneal injections of the test substance following infection. Animals in group *C* received daily injections of the test substance for four days before infection only.

TABLE 4

SURVIVAL TIME OF SWISS ALBINO MICE INFECTED WITH VALLÉE STRAIN OF TUBERCLE BACILLI AND TREATED WITH PROTEIN FRACTIONS OF HUMAN SERUM

Group	Number	50 Per Cent Survival Time	Mean Survival Time
		days	*days*
A—saline	12	38.0	38.7
B—tuberculous serum	22	36.5	38.8
C—tuberculous serum fractions II–III—10%	23	41.0	40.0
D—tuberculous serum fraction IV—10%	24	48.0	44.2
E—10% immune gamma globulin	18	35.0	35.7

Animals received four daily intraperitoneal injections of the test substance before infection only.

given gamma globulin (commercial). It should be noted that, for *Experiments 1* and *2*, the gamma globulin used was a 16.5 per cent solution whereas, for *Experiment 3*, it was diluted with saline in order to make it a 10 per cent solution (table 4).

Discussion

These preliminary experiments are presented as suggestive evidence that there exist, in two fractions of human serum, substances which may afford protection against tuberculous infection. They represent, to the knowledge of the present writers, the first evidence that fractions of serum may contain protective factors against this disease.

The difficulty in the past of demonstrating the presence of protective humoral antibodies in tuberculosis may be due to several factors. First, there may be no protective antibodies present. The writers do not believe that this is the case. Rich (7), in a comprehensive discussion of acquired resistance to tuberculosis, presents the evidence and concludes that it "leaves no doubt that infection with virulent tubercle bacilli, or vaccination with attenuated or killed bacilli gives rise to a definite degree of acquired resistance in experimental animals; and there is excellent evidence that the same is true of the human being." The present writers believe that the presence of acquired resistance in the entire body, at sites distant from as well as close to the original locus of infection, is good evidence for the presence of a humoral factor.

Second, the antibody titer may be low. If this is true, it would be necessary to concentrate the source in order to demonstrate its presence. Third, the antibody may be closely bound to antigen and therefore not available. If this is the case, its protective effect could not be demonstrated unless the complex could be split. Fourth, antibody in one fraction of serum may be interfered with or inhibited by a substance in another serum fraction. The work of Cole and Favour (8) suggests that this may occur. In their experiments on passive transfer of tuberculin and PPD sensitivity, they found that Cohn fraction II may inhibit the action of antibody found in fraction IV-10. The work of Gerstl and his associates (9, 10) also suggests this possibility. The latter group found that fractionating serum before testing for antibody, by means of hemagglutination and complement fixation tests, could "unmask" antibody activity which could not be detected in whole serum. Seibert and her associates (11), in a recent paper, have demonstrated that tuberculopolysaccharide and, to a lesser extent, tuberculoproteins *A* and *B* interfere *in vitro* and *in vivo* with antigen-antibody reactions of the serum of rabbits immunized with BCG.

With the new methods of fractionating and concentrating serum available, it may become possible to isolate protective antibodies which were previously elusive because of such factors as those mentioned above. It was most interesting to find in these experiments that there was an increase in survival time with Cohn fractions II-III and IV of tuberculous serum. The suggestive evidence for a protective effect of fraction IV is particularly noteworthy because antibodies have not been demonstrated previously in this fraction. The possible protection given

by immune gamma globulin (commercial) suggests that there may be protective antibodies in pooled human sera due to past infection, followed by continued antibody production.

The work reported here is being repeated with larger groups of mice as well as with guinea pigs. Total bacterial population studies of mouse lungs and spleens using the technique of Fenner and associates (12) are being done.

Summary

Preliminary experiments are described in which fractions of serum from tuberculous patients, whole tuberculous serum, and immune gamma globulin (commercial) were found to inhibit the growth *in vitro* of the H37Rv strain of tubercle bacilli. The same test substances increased, to varying degrees, the survival time of mice infected with the Vallée strain of tubercle bacilli.

Sumario

Experimentos Preliminares sobre el Pase Pasivo de Anticuerpos Humorales Protectores en la Tuberculosis

Descríbense experimentos preliminares en que se descubrió que fracciones del suero de tuberculosos, el suero tuberculoso íntegro y la inmuniglobulina gamma (comercial) inhibían la proliferación *in vitro* de la cepa H37Rv de los bacilos tuberculosos. La misma substancia de ensayo acrecentó, en mayor o menor grado, el tiempo de sobrevivencia de los ratones infectados con la cepa Vallée del bacilo tuberculoso.

Resume

Expérimentations préliminaires sur le transfert passif d'anticorps humoraux dans la tuberculose

Description d'expérimentations préliminaires au cours desquelles des fractions de sérum provenant de malades tuberculeux, de sérum tuberculeux total et de gamma-globuline immune (commerciale) ont démontré *in vitro* leur activité inhibitrice sur la souche H37Rv du bacille tuberculeux. Ces mêmes substances de test augmentaient à divers degrés les temps de survie des souris infectées avec la souche Vallée du bacille tuberculeux.

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EFFECT OF ISONIAZID AND CORTISONE IN CHILDREN VACCINATED WITH BCG[1, 2]

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INTRODUCTION

In a previous paper, the administration of isoniazid in children vaccinated with BCG was reported (1). At that time it was concluded that this resulted in inhibition of the suppurative reaction at the site of vaccination; inhibition of allergic manifestations in terms of failure to become tuberculin positive after BCG vaccination; modification of the histology of the site of vaccination; minimal influence on the Middlebrook-Dubos agglutination titer; and an unaltered serum protein electrophoretic pattern.

The above changes were further influenced by the addition of cortisone. The present paper continues this work, with particular emphasis on the histologic changes.

MATERIAL AND METHODS

Twenty-four children between the ages of six and eleven were included in this study. The children had negative Mantoux readings to 10 units of PPD tuberculin four days prior to the study. They were vaccinated intradermally with BCG into the left deltoid area.

The children were divided at random into three groups of 8 each. *Group 1* was used as a control group. *Group 2* was given isoniazid daily at a dosage of 4 to 6 mg. per kg. of body weight. *Group 3* was given isoniazid daily (4 to 6 mg. per kg. of body weight) as well as cortisone, 0.5 mg. per kg. of body weight daily.[3]

In both groups treatment was started simultaneously with the vaccination.

Every two weeks up to the eighth week following vaccination the primary skin lesion from 2 children in each group was excised and examined histologically. A careful search for acid-fast bacilli was made. At the same time Mantoux reactions, Middlebrook-Dubos agglutination tests, and serum protein electrophoretic patterns were performed every two weeks on the entire group of children. The latter studies were all carried out until the end of the tenth week. Treatment with isoniazid and/or cortisone was discontinued on the day of biopsy, but was continued in the children who had not had biopsies for the full ten weeks until the end of the study.

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[1] From the Pediatric Clinic, Athens University, Athens, Greece.

[2] The original manuscript, submitted to the REVIEW by Dr. Choremis and his associates, was a more detailed presentation of the observations described in the present report. Because of space limitation, it was not possible to publish the manuscript as originally submitted. Accordingly, with the permission of Dr. Choremis and his associates, the original manuscript was turned over to Dr. Gerald E. Muehsam of the Veterans Administration Hospital, East Orange, New Jersey, for condensation. Both the authors of the manuscript and the editor of the REVIEW wish to express their appreciation to Dr. Muehsam for his very considerable contribution to the publication of this report.—*The Editor*

[3] Generously supplied as Deltacortril by Chas. Pfizer & Co.

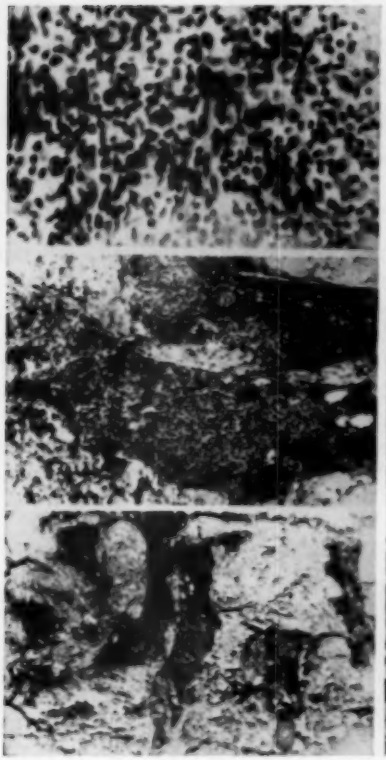

FIG. 1

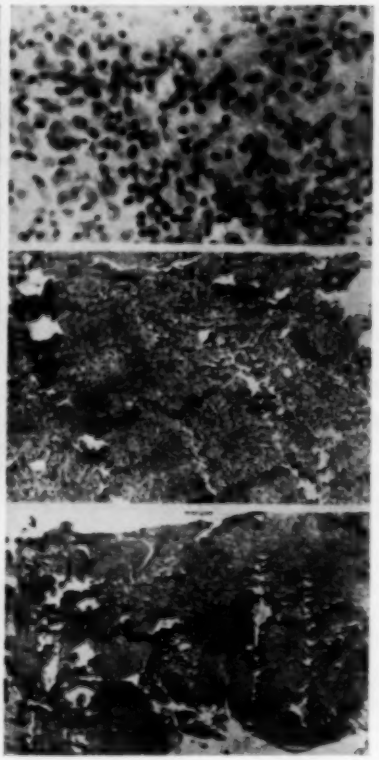

FIG. 2

FIG. 1. *Group 1*, control group (untreated cases): Excision two weeks after vaccination shows minimal infiltration of epidermis, but extensive lymphocytic infiltration of the dermis and subcutaneous fat. There is no caseation or tubercle formation.

FIG. 2. *Group 1*, control group, four weeks after vaccination, shows ulceration of epidermis with dense collections of leukocytes in dermis and early epithelioid cell formation. No tubercles are seen.

RESULTS

Clinical

Although complete follow-up of the development of the primary skin lesion was not possible, because some of the lesions were removed surgically, the observations up to the date of biopsy led to the conclusion that the effect of the administered drugs was similar to that previously observed (1).

Immunologic Studies

Mantoux reaction: In *Group 1* (untreated cases), the Mantoux reaction became positive in all 8 children (10 units of PPD intradermally).

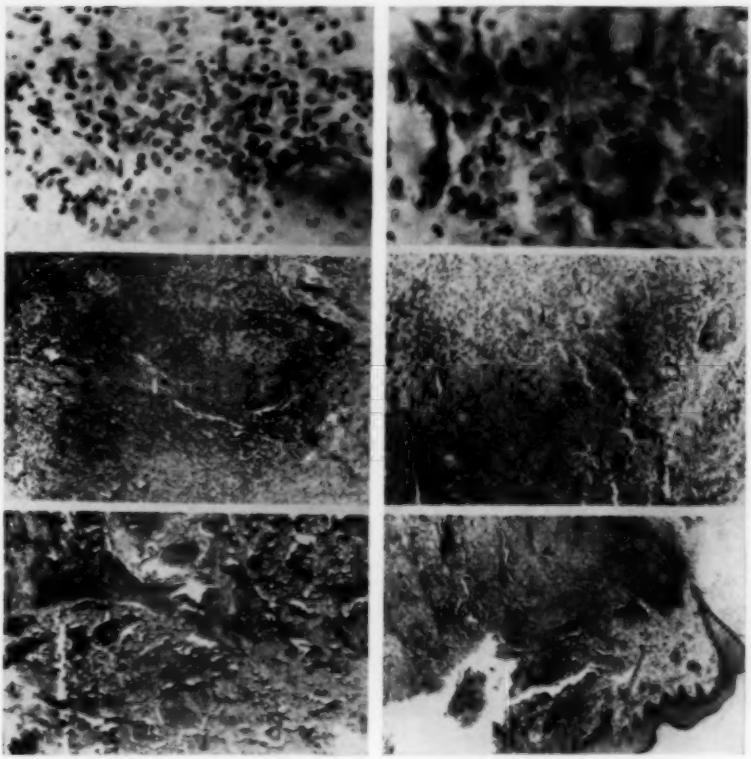

FIG. 3 FIG. 4

FIG. 3. Control group, aged six weeks, shows hyperkeratosis and infiltration of the epidermis and beginning epithelioid tubercle formation. There is no caseation.

FIG. 4. Control group, aged eight weeks, shows definite epithelioid giant cell tubercles of the dermis without caseation. The epidermis is relatively clear.

In *Group 2* (isoniazid-treated children), a positive reaction was observed in 2 children after two weeks of treatment and in one child after six weeks of treatment. The remaining 5 children remained Mantoux negative for the entire ten weeks of the study.

In *Group 3* (isoniazid- and cortisone-treated children), a positive Mantoux reaction was present only in the 2 children in whom treatment was discontinued after two weeks. The remaining 6 children continued to be Mantoux negative.

The agglutination titer was present in all three groups, but there was no significant difference between control and treated groups.

The electrophoretic patterns of serum proteins remained within the normal range in all three groups.

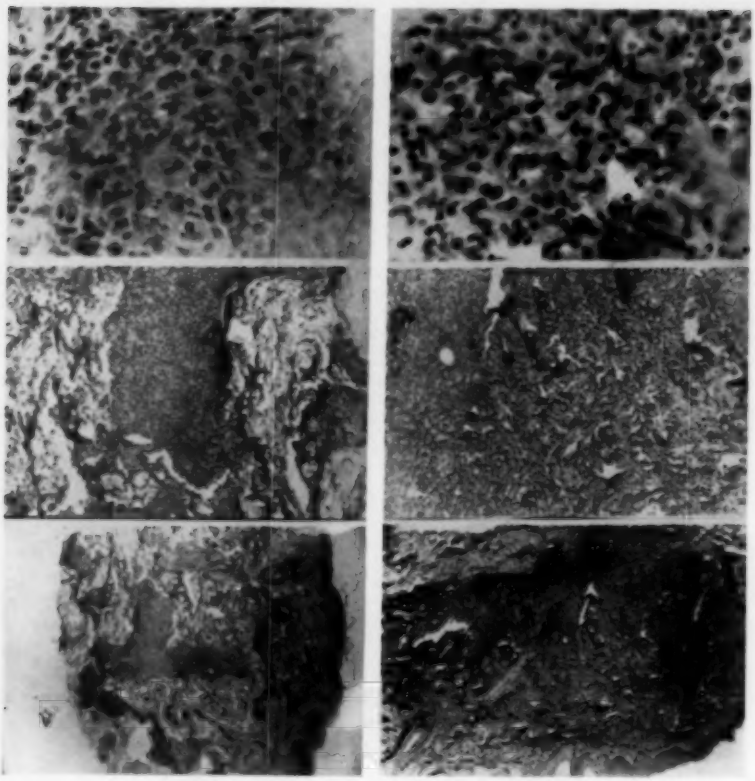

FIG. 5 FIG. 6

FIG. 5. *Group 2.* Isoniazid-treated patient two weeks following vaccination shows a normal epidermis with dense lymphocytic and leukocytic infiltration and early epithelioid cell transformation. No caseation or tubercle formation.

FIG. 6. *Group 2* (isoniazid-treated) four weeks after vaccination shows a normal epidermis with dense collections of lymphocytes, leukocytes, and epithelioid cells as well as fibroblasts. The infiltrate suggests a tuberculous granuloma, but there is no caseation or giant cell formation.

Histologic Studies

There were minor differences in the histologic picture of individual cases within each group, but an attempt is made in this summary to point out only the main differences.

In *Group 1* (untreated cases), the specimens showed an extensive diffuse, dense cellular infiltrate of the epidermis, dermis, and subcutaneous fat. The epidermis showed vesicle formation and, in one case, ulceration. The cellular infiltrate initially was exudative in character, but subsequently showed suppuration with-

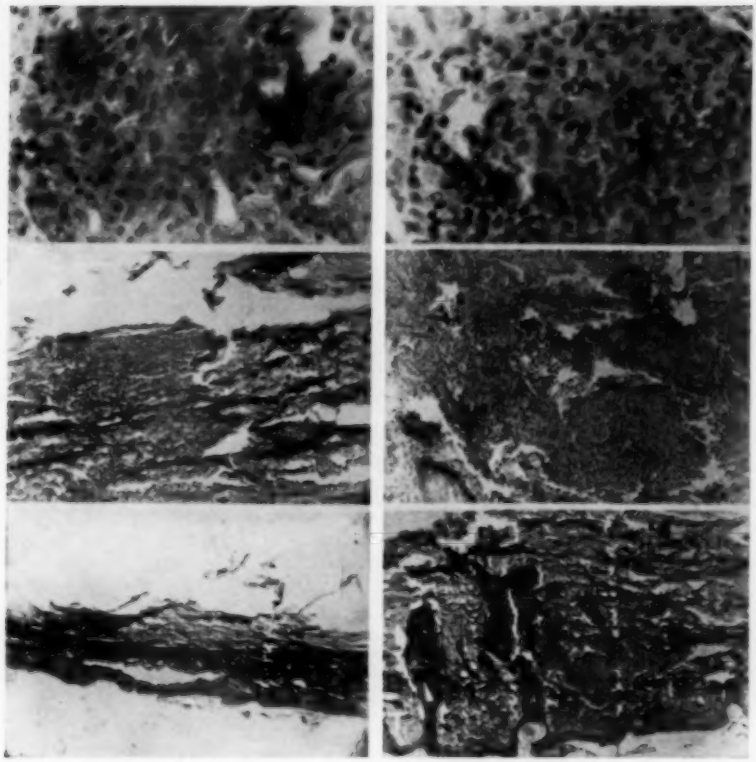

FIG. 7 FIG. 8

FIG. 7. *Group 2* (isoniazid-treated) six weeks following vaccination shows normal epidermis and a moderately dense infiltration in the dermis without caseation or tubercle formation.

FIG. 8. *Group 2* (isoniazid-treated) eight weeks following vaccination shows normal epidermis with continued lymphocytic, epithelioid, and fibroblastic infiltration of the dermis, but no caseation or giant cell formation.

out caseation and progressed through an epithelioid cell stage into a full-blown typical tuberculous lesion by the end of the eighth week.

In *Group 2* (isoniazid-treated cases), the specimens showed no infiltration of the epidermis. The dermis, however, was extensively infiltrated, with the development of an epithelioid cell reaction. This was suggestive, but not diagnostic, of tuberculosis because of the absence of caseation and giant cell tubercles. The lesions had not subsided by the end of the eighth week.

Thus, it appeared that the development of full-blown tuberculous lesions was inhibited by treatment.

Group 3 (isoniazid plus cortisone treatment) again showed sparing of the

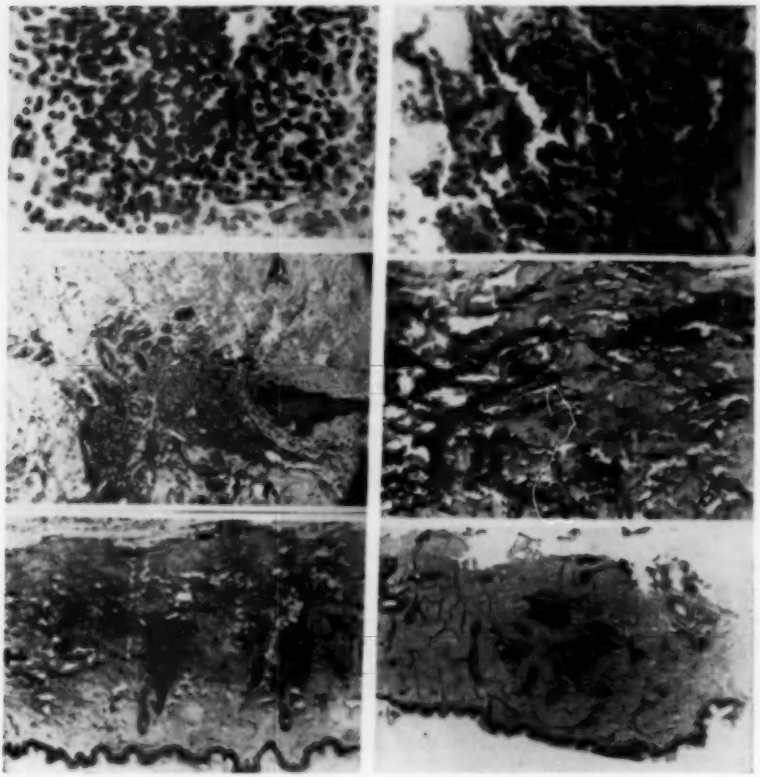

FIG. 9 FIG. 10

FIG. 9. *Group 3* (combined treatment) two weeks after vaccination shows a normal epidermis, a patchy, nonspecific infiltrate in the dermis, and there are no tubercles present.

FIG. 10. *Group 3* (combined treatment) four weeks after vaccination shows a normal epidermis and a scant lymphocytic infiltrate with a few fibroblasts and leukocytes. There are no epithelioid cells.

epidermis. The dermis here showed a patchy, nonspecific, predominantly lymphocytic infiltration which was much less extensive than it was in the other two groups. Epithelioid cells were not observed and there was a tendency to almost complete healing with fibroblastic proliferation by the end of the eighth week. At that time the initial cellular infiltrate had almost completely disappeared.

It thus appears that in this group there was even further inhibition of tubercle formation than in the second group.

DISCUSSION

The antiallergic effect of isoniazid alone or in combination with cortisone is again confirmed by this study. This is suggested by the fact that only 3 of 8

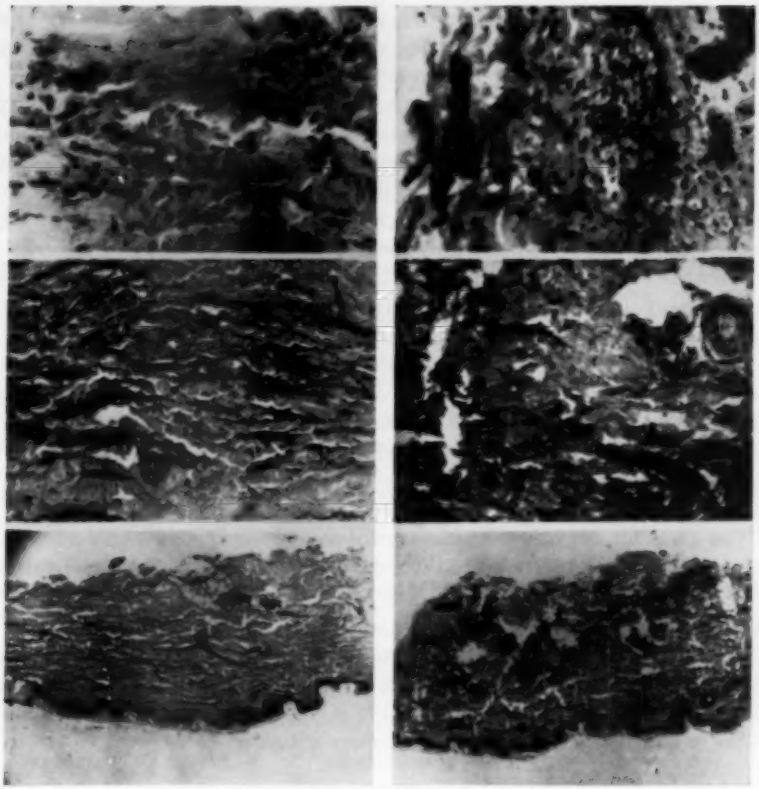

FIG. 11 FIG. 12

FIG. 11. *Group 3* (combined treatment) six weeks following vaccination shows a normal epidermis with few lymphocytic infiltrations, but no epithelioid cells or tubercle formations.

FIG. 12. *Group 3* (combined treatment) eight weeks following vaccination shows a normal epidermis with minimal lymphocytic infiltration and increase in fibrous tissue proliferation.

children treated with isoniazid showed positive Mantoux reactions. Two of these children had had treatment discontinued after two weeks, one after six weeks, coinciding with the time of excision of the vaccination site. The combined treatment group (isoniazid and cortisone) contained only 2 children with positive Mantoux reactions and 6 with no reaction. In contrast to this, all children of the control group (untreated) reacted positively to tuberculin skin tests. The conversion, regardless of group, took place six to eight weeks following the vaccination.

It does not appear that excision of the primary lesion influences the course of allergic manifestation as long as this excision is performed at least two weeks following vaccination.

The Middlebrook-Dubos reaction is not significantly influenced by either the administration of drugs or the excision of the primary lesion. Similarly, the serum protein electrophoretic pattern is not altered by the manipulation.

Administration of isoniazid alone, following the administration of BCG vaccine, inhibits the full development of typical tuberculous lesions, resulting in an almost nonspecific inflammatory reaction. Furthermore, combined treatment with isoniazid and cortisone results in an entirely nonspecific inflammatory reaction which is less pronounced and more localized, with more rapid healing, so that there is almost complete healing by the end of the eighth week.

From the above data it is concluded that the inhibitory effect of isoniazid on the development of tuberculous lesions is further augmented by the administration of cortisone.

Since suppuration of the primary lesion, which is observed only in nontreated cases, precedes the full development of tuberculous lesions by a considerable time interval, it is assumed that suppuration does not have any relationship to caseation. It is difficult to understand why children who are under treatment with the above-mentioned drugs following BCG vaccination do not develop suppuration.

The available literature reports work done only on experimental animals or tuberculous patients, so that it is difficult to directly compare the present studies with those of other investigators. This is particularly true in reference to the histologic findings of the primary skin lesions with or without treatment.

In conclusion, it is believed that combined administration of isoniazid and cortisone has a beneficial effect in the treatment of tuberculosis. The present writers are now investigating the question of whether this beneficial effect is due to the antiallergic and/or anti-inflammatory action of cortisone.

Summary

BCG was administered intradermally to 24 children between the ages of six and eleven years. The children were divided into three groups of 8 each. One group served as controls. All of the children became Mantoux positive and, on biopsy of the vaccination site, demonstrated typical tuberculous lesions. The second group received isoniazid from the day of vaccination. Three children of this group became Mantoux positive. All of the 8 children, however, on skin biopsy, displayed epithelioid cell infiltration of the dermis but absence of caseation and giant cell formation.

The third group received both isoniazid and cortisone. Two became Mantoux positive and the entire group, on biopsy, demonstrated only nonspecific lymphocytic infiltration of the dermis.

These observations suggest that the inhibitory effect of isoniazid on the development of tuberculous lesions is further augmented by the administration of cortisone.

Sumario

Los Efectos de la Isoniacida y la Cortisona sobre los Niños Vacunados con BCG

A 24 niños de seis a once años de edad se les administró intradérmicamente BCG. Los niños fueron divididos en tres grupos de 8 cada uno. Un grupo sirvió de testigo. Todos los niños se volvieron positivos a la Mantoux, y al hacer la biopsia del sitio vacunado, mostraron típicas lesiones tuberculosas. El segundo grupo recibió isoniacida desde el día de la vacunación. Tres niños de este grupo se volvieron Mantoux-positivos. No obstante, todos los ocho niños, en la biopsia cutánea, revelaron infiltración por células epitelioideas de la dermis, pero falta de caseación y de formación de células gigantes.

El tercer grupo recibió tanto isoniacida como cortisona. Dos de los niños se volvieron Mantoux-positivos, y ninguno del grupo reveló, en biopsia, más que infiltración linfocítica anespecífica de la dermis.

Sugieren estas observaciones que el efecto inhibidor de la isoniacida sobre la producción de lesiones tuberculosas se ve aun más reforzado por la administración de cortisona.

Resume

Action de l'isoniazide et de la cortisone chez les enfants vaccinés par le BCG

Après administration intradermique de BCG chez 24 enfants âgés de six à onze ans, les enfants furent répartis en trois groupes de 8 enfants. Un groupe servait de témoin. La réaction de Mantoux a été positive chez tous les enfants, et la biopsie effectuée au point vaccinal a révélé la présence de lésions tuberculeuses. Le deuxième groupe a reçu de l'isoniazide à partir du jour de la vaccination. Trois enfants du groupe ont présenté une réaction de Mantoux positive. Chez tous les huit enfants du groupe, la biopsie effectuée au point vaccinal a révélé l'infiltration du derme par des cellules épithélioïdes, sans caséation ou formation de cellules géantes.

Le troisième groupe a reçu de l'isoniazide et de la cortisone. Deux réactions de Mantoux positives furent observées et, chez tous les enfants, la biopsie a uniquement révélé l'infiltration lymphocytaire du derme.

Ces observations suggèrent que l'action inhibitrice de l'isoniazide sur le développement des lésions tuberculeuses est encore accrue par l'administration de cortisone.

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NOVOBIOCIN[1]

Activity *in Vitro* and in Experimental Tuberculosis

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INTRODUCTION

Novobiocin is a new antimicrobial produced from *Streptomyces spheroides* (1). It was found to be inhibitory *in vitro* against a wide variety of gram-positive and occasional strains of gram-negative bacteria (2–5). Growth of *Mycobacterium tuberculosis* of human origin in Proskauer and Beck medium was inhibited by 5 to 10 γ of the drug per ml., but it did not prove effective in the treatment of mice experimentally infected with tubercle bacilli.

In the early phase of its development, novobiocin was made available to the present writers for further investigation of its antituberculous activity *in vitro* and *in vivo* in guinea pigs. The present report describes the effect of novobiocin on a variety of virulent, attenuated, and avirulent mycobacterial strains grown on different culture media. Data on the emergence of novobiocin-resistant tubercle bacilli and on novobiocin treatment of experimentally produced tuberculosis in guinea pigs are also presented.

MATERIALS AND METHODS

The novobiocin[2] used throughout was the crystalline monosodium salt, dissolved in physiologic sodium chloride solution and sterilized by passage through a Seitz filter. The *in vitro* activity of the drug against mycobacteria was tested in Tween® albumin, Proskauer and Beck and oleic acid liquid media, and on nutrient blood agar and Hohn's egg medium.

The following mycobacteria were included in the study:

Virulent tubercle bacilli: 31 strains recently isolated from sputum. Some of these were resistant to streptomycin, isoniazid or para-aminosalicylic acid (PAS) or to combinations of these drugs. Stock strains, including H37Rv, were also studied.

Avirulent variants of human tubercle bacilli: 3 strains.

BCG: 3 strains.

A virulent bovine strain (Ravenel); and an *avian strain.*

Photochromogenic acid-fast bacilli: 15 strains, isolated from sputum, apparently the cause of pulmonary disease.

Nonchromogenic acid-fast bacilli: 30 strains isolated from sputum, soil, or sewage. Some of these were isolated in this laboratory and the others elsewhere.

The mycobacteria were grown in Tween-albumin medium for ten to twelve days, and the growth was diluted 10^{-1}. The novobiocin susceptibility studies were carried out by inoculating 5 ml. quantities of the liquid test media, containing different concentrations of the drug, with 0.1 ml. of the diluted culture. In the case of the rapidly growing strains, one loopful of the diluted culture was used for inoculum. In the tests on solid media seeding was carried out with a loop also. Final readings of the drug-susceptibility tests in liquid and solid media were made at fourteen days and twenty-eight days, respectively.

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[1] From the Ray Brook State Tuberculosis Hospital, Ray Brook, New York.

[2] Generously supplied as Cathomycin by Merck and Co., Inc.

Novobiocin assay was performed by the agar diffusion method using *Micrococcus pyogenes* var. *aureus* (Smith) as the test organism and nutrient agar as the test medium.

For convenience, details of the *in vivo* studies are given with the report on these experiments.

Observations

Studies in Vitro

Novobiocin in a concentration of 4 γ per ml. in Tween-albumin medium completely inhibited, for fourteen days, growth of the human tubercle bacilli studied. There was partial inhibition by 2 γ of the drug per ml. This finding applied whether the tubercle bacilli were resistant or susceptible to streptomycin, isoniazid, or PAS.

Complete inhibition of one of the avirulent strains (H4Ra) required 10 γ of novobiocin per ml. An avian strain and the Ravenel bovine strain were found to be as susceptible to the drug as the human strains. The BCG strains, however, showed some growth in the presence of 10 γ of the drug per ml. It is interesting to note that most of the photochromogenic and nonchromogenic acid-fast strains were able to grow in 10 to 100 γ of novobiocin per ml.; only 4 of the 35 strains studied were completely inhibited by 4 γ per ml.

In Proskauer and Beck medium, the range of complete inhibition of tubercle bacilli was 5 to 10 γ of novobiocin per ml. The drug was least active against tubercle bacilli in oleic acid liquid medium; good growth occurred in the presence of 40 γ per ml. and there was only partial inhibition by 50 γ per ml. This was apparently not due to inactivation of the drug by the medium, as repeated assay of different concentrations of novobiocin in oleic acid medium showed no loss of activity against the test staphylococcal strain during fourteen days of incubation. There was appreciable loss of activity after twenty-one days of incubation, but similar loss was also noted in aqueous solutions of novobiocin stored in the refrigerator for this period of time.

To determine whether novobiocin would remain stable during inspissation, serial dilutions, ranging from 0.125 to 4 γ per ml. were prepared in tryptose phosphate broth and were subjected to a temperature of 91°C. for ninety minutes. Subsequent agar diffusion assay revealed no appreciable loss of activity.

As novobiocin activity was not affected by inspissation temperature, its effect on the growth of tubercle bacilli on solid medium could be determined. The drug was added, in concentrations ranging from 5 to 25 γ per ml., to nutrient blood agar and to Hohn's egg medium prior to inspissation. On these media tubercle bacilli were only partially inhibited by 25 γ per ml., the highest concentration of drug employed.

Emergence of novobiocin-resistant strains: In the first experiment the mutation pattern of *Micrococcus pyogenes* var. *aureus* was studied by the surface plating method. Agar plates containing 1, 3, 10, and 100 γ of novobiocin per ml. were each inoculated with 1 ml. of a broth culture of the original parent strain. This inoculum contained approximately 300 million living cells.

An average of 17 colonies of the test organism grew out on each of the ten plates containing 1 γ of novobiocin per ml. There was no growth on the plates containing higher concentrations of the drug. Several of the resistant colonies, selected at random for further analysis, were grown in broth without drug. When the desired growth was obtained the cells were plated on the same concentrations of novobiocin. The inoculum for the plates containing 1 γ per ml. was diluted in order to obtain isolated colonies. As expected, almost 100 per cent of the cells were resistant to 1 γ of novobiocin per ml. Mutants resistant to higher concentrations were also present in these populations resistant to 1 γ per ml., as shown by an average of 150 colonies growing on plates containing 3 γ per ml. and an average of 27 colonies present on plates containing 10 γ per ml. There was no growth on plates containing 100 γ per ml.

This plating procedure was applied to colonies resistant to 10 γ per ml. and it was again demonstrated that variants resistant to higher concentrations of drug were present in certain numbers. Eventually it was possible, by this method, to select cells resistant to 100 γ of novobiocin per ml.

The development of novobiocin-resistant tubercle bacilli, strain H37Rv, was accomplished by growing the bacilli in increasing concentrations of the drug in Tween-albumin medium. The parent strain was first inoculated in medium containing 1 γ per ml. and, when good growth was obtained, it was transferred to medium containing a higher concentration of drug. This process was repeated until finally, after seven transfers, the resulting strain was capable of growth in 50 γ of novobiocin per ml.

Studies in Vivo

Four experiments were carried out at different times. Inbred albino guinea pigs weighing 750 to 800 gm. were infected subcutaneously in the groin with virulent tubercle bacilli, strain H37Rv. The inoculum was 0.5 ml. of a ten-day-old Tween-albumin liquid culture diluted 10^{-4}. The animals were randomly separated into two groups: one group to receive immediate treatment with novobiocin and one to serve as untreated controls.

Fresh aqueous solutions of novobiocin were prepared daily and were administered either subcutaneously or orally. Details concerning the dosage, mode, and duration of treatment are given separately with the results of each experiment.

Experiment 1: Twenty guinea pigs were infected with virulent tubercle bacilli; 10 received a daily novobiocin dose of 100 mg. per kg. subcutaneously in the abdomen, and 10 remained untreated.

It was obvious at once that subcutaneous injection of this dosage was painful to the animals. The pH of the solution was not considered responsible since it was near neutrality.

The first treated animal died on the seventh day and the others were listless and ate poorly. By the twelfth day half of the treated animals had died. There were no gross tuberculous lesions in these animals or in 2 untreated animals killed at this time. The common necropsy finding in the treated animals was necrosis of the subcutaneous tissues of the abdomen which eroded into the abdominal cavity and resulted in extensive diffuse adhesion of the viscera.

Treatment was discontinued on the twelfth day because the animals were so

ill. On the twenty-fifth day of the experiment the remaining animals were killed: 2 in the treated group and 8 controls. The treated animals had tuberculous abscesses at the site of infection. The spleen and liver were of normal size and showed no gross lesions, but sections of the tissues revealed microscopic evidence of tuberculosis. The control animals showed widespread tuberculosis. The spleens were all greatly enlarged and disease was present in varying degrees in spleen, liver, and lungs.

Although it was evident soon after the start of this experiment that 100 mg. per kg. of novobiocin were extremely toxic, both locally and generally, there appeared to be a definite suppressive effect on the progression of tuberculosis in the 2 guinea pigs which survived for twenty-five days.

Experiment 2: Eighteen guinea pigs were infected with virulent tubercle bacilli; 9 received novobiocin subcutaneously in a daily dosage of 25 mg. per kg., and 9 remained untreated.

At the end of one week there was no evidence of toxicity, but the injection sites were thickened and inflamed. In some cases this progressed to ulceration and scab formation. Enlargement of the lymph nodes at the site of infection was observed in the animals of both groups.

The first evidence of toxicity appeared on the twelfth day: the treated animals were eating poorly, had lost weight, and were less active than the controls. One death occurred at this time. There was a large tuberculous abscess at the infection site and no gross lesions in the internal organs although a few acid-fast bacilli were found on films prepared from the liver and spleen. A control animal was killed for comparison; it was in good nutrition and there were no gross lesions other than an abscess in the groin.

Two treated animals died on the twenty-fifth day and 2 control animals were killed for comparison of the findings. All had tuberculous abscesses in the groin and slight gross evidence of tuberculosis. The gross findings in the internal organs were essentially similar in both the treated and untreated animals. It was believed that the three deaths in the treated group were due to some toxic effect of novobiocin and not to tuberculosis.

On the thirty-fourth day one animal from each group was killed, and widespread tuberculosis was observed in both. It was therefore decided to terminate the experiment on the fortieth day. Five animals remained in each group. The control animals were in good nutrition and appeared healthy, while the treated animals were sluggish and had lost considerable weight. All had abscesses at the site of infection; these were draining in the treated animals. All of the animals, treated and untreated, had numerous isolated and confluent tubercles in the spleen, liver, and lungs, and the spleens were greatly enlarged. There was no significant difference in the findings in the two groups.

It was concluded from this experiment that novobiocin in a dosage of 25 mg. per kg. daily has no suppressive effect on tuberculosis in the guinea pig and, further, that this dosage has a definite local and systemic toxic effect although this is considerably less than that produced by 100 mg. of the drug per kg.

Experiment 3: In this study novobiocin in aqueous solution was administered

orally in a dosage of 150 mg. per kg. of body weight. Ten guinea pigs were infected with virulent tubercle bacilli and treatment was begun at once in 5; the remaining 5 served as untreated controls.

On the tenth day there was nodular enlargement with abscess formation at the site of infection in the animals of both groups. The area of involvement was considerably smaller in the treated animals. The first evidence of toxicity appeared after sixteen days, and on the nineteenth day one of the treated animals died. A 3 by 4 mm. abscess in the groin which contained pus and tubercle bacilli was noted; the internal organs were grossly normal, and only a few acid-fast bacilli could be found in films of the spleen. An untreated animal killed for comparison had a large granulomatous area, measuring 2 by 3 cm., in the groin, and there was widespread tuberculosis of the liver and spleen, and a few tubercles were found in the lungs.

Novobiocin treatment was stopped at this time because the animals were obviously ill. On the twenty-first day 2 animals died and the remaining 2 were in such poor condition that the experiment was terminated.

All of the treated animals presented an essentially similar picture. There were abscesses at the site of infection, and tubercle bacilli could be readily demonstrated in their contents. The spleens and lungs were grossly normal and the livers showed one or two small tuberculous foci. Films of the organs were carefully searched for acid-fast bacilli. Only a very rare bacillus (1 to 6 on a slide) could be found on films of the spleens of 3 of the treated animals.

The remaining 4 control animals showed large abscesses in the groin and enlarged spleens with tubercles of varying size. The liver of one animal showed only a few isolated tubercles while in the other animals the livers were literally peppered. Only isolated tubercles could be seen grossly in the lungs.

There was unquestionable difference between the treated and untreated animals in the extent of tuberculous disease. It is apparent, from these findings and those of the first experiment, that, when sufficiently large doses of novobiocin are used, experimental tuberculosis in the guinea pig is definitely suppressed. Unfortunately, these large doses are much too toxic for prolonged therapy.

Experiment 4: Ten of twenty guinea pigs infected with virulent tubercle bacilli were treated with a daily oral dose of 75 mg. per kg. of novobiocin.

On the thirteenth day all of the animals had nodular enlargement with abscess formation at the site of infection. The involved area was 2 by 1 cm. or larger; this was considerably greater than that observed in the preceding experiment.

Treatment with novobiocin was continued for thirty-five days, at which time the treated and untreated animals were killed. None had died during the experiment and, furthermore, there was no evidence of drug toxicity.

All of the control animals showed extensive tuberculosis in the liver and spleen. The spleens were enlarged two to four times normal size. There were isolated disease foci in the lungs.

There was disease in the liver, spleen, and lungs of the treated animals but to a lesser degree than was seen in the untreated animals. In 4 animals the spleen

was of almost normal size but a few pinpoint tubercles could be seen. The extent of disease in one treated animal was roughly comparable to that of the control animal with the least extensive involvement.

The findings in this experiment indicate that, while there was no evidence of toxicity with a smaller dosage of novobiocin, its suppressive effect on tuberculosis in the guinea pig was likewise reduced.

Discussion

In vitro experiments show that the tuberculostatic effect of novobiocin is dependent upon the test medium. In Tween-albumin medium the growth of virulent strains of *Mycobacterium tuberculosis* is inhibited by 4 γ of the drug per ml. while 50 γ per ml. only partially inhibit growth of these strains in oleic acid liquid medium. The reason for this is not clear. Agar diffusion assay, using *Micrococcus pyogenes* var. *aureus* as the test organism, revealed that novobiocin was not inactivated by oleic acid medium.

The emergence of microorganisms resistant to novobiocin occurs readily although mutants highly resistant to the drug cannot be obtained in one step, as with streptomycin, from a susceptible parent culture of *Micrococcus pyogenes* var. *aureus*. Highly resistant cells must be selected from the bacterial populations of colonies growing in the presence of lower drug concentrations.

Most of the "atypical" and saprophytic mycobacterial strains studied are highly resistant to novobiocin and, consequently, this drug can be used profitably to help to differentiate these strains from tubercle bacilli.

The tuberculostatic effect of novobiocin in experimentally infected guinea pigs is slight with dosages that can be tolerated. The effect of the drug is more pronounced when higher dosages are used, but these proved extremely toxic for the animals. Manifest toxicity is less when the drug is given orally.

Summary

Novobiocin completely inhibits the growth of virulent strains of *Mycobacterium tuberculosis* in a concentration of 4 γ per ml. in liquid Tween®-albumin medium. The drug is considerably less effective when certain other media are used. Tubercle bacilli resistant to novobiocin emerge readily. Under certain experimental conditions novobiocin exerts a slight to moderate suppressive effect on the progression of tuberculosis in the guinea pig.

Sumario

La Novobiocina: Actividad in Vitro y en la Tuberculosis Experimental

La novobiocina inhibe absolutamente la proliferación de cepas virulentas del *Mycobacterium tuberculosis* a una concentración de 4 γ por ml. en el medio de Tween®-albúmina líquido. La droga es mucho menos eficaz cuando se usan otros medios. Aparecen fácilmente bacilos tuberculosos resistentes a la novobiocina. En ciertas condiciones experimentales, la novobiocina ejerce un efecto supresor que varía de leve a moderado sobre la agravación de la tuberculosis en el cobayo.

Resume

Novobiocine: Actirité in vitro et Tuberculose expérimentale

La Novobiocine à la concentration de 4 γ par ml en milieu Tween-albumine inhibe complètement la croissance de la souche virulente du *Mycobacterium tuberculosis*. La drogue est considérablement moins efficace lorsque certains autres milieux sont employés. Les bacilles tuberculeux novobiocine-résistants apparaissent facilement. Dans certaines conditions expérimentales, la novobiocine exerce une action suppressive légère ou modérée sur la progression de la tuberculose du cobaye.

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SURFACE ALTERATION STUDIES OF MYCOBACTERIUM TUBERCULOSIS WITH TRYPSIN[1, 2]

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(Received for publication November 4, 1955)

INTRODUCTION

In the search for chemotherapeutic agents in tuberculosis, recent investigations indicate the value of the enzyme pancreatic trypsin,[3] as an adjunct to the beneficial effects of antimicrobial drugs in tuberculous empyema (1). For example, pancreatic trypsin has been successfully administered intrapleurally for the sterilization of disease processes and re-expansion of the lung in selected cases of tuberculous fibrinopurulent empyema. Among the noteworthy beneficial effects accompanying trypsin therapy is the repeated failure to demonstrate tubercle bacilli by microscopy, culture, or animal inoculation from known infected pleural tissue fluids of the intrapleural lavage. This phenomenon has been observed in fluids which were shown to be positive prior to trypsin lavage (1). This *in vivo* phenomenon seemed worthy of investigation *in vitro*. Trypsin-treated tubercle bacilli therefore were observed first for surface modifications.

METHODS AND MATERIALS

Preparation of Trypsin-Treated Cells

Mycobacterium tuberculosis (H37Rv) was grown for approximately twenty-eight days in 250 ml. flasks containing 50 ml. of Dubos Tween® 80 liquid medium, with 1 per cent glycerol. The bacterial suspensions were pooled in large centrifuge bottles and kept at refrigerator temperature for approximately two to five days (table 1). Acid-fast and Gram stains were prepared and media was inoculated from the sedimented cells. Staining was incorporated to confirm the presence of mature cells. Bacterial contamination was checked with nutrient agar and thioglycollate broth. Petragnani slants were cultured to identify corded colonies and Dubos media for LD_{50} determinations. The supernatant was removed and the pooled cells were washed three times in 50 ml. Rockefeller centrifuge tubes with sterile phosphate buffered saline, pH 7.8. The cells were then divided into two equal portions, each containing 1 ml. of packed cells. The cells were dispersed, diluted, nitrogen determinations were completed (2), and Dubos oleic-acid–albumin agar medium was inoculated for plate counts. Buffered physiologic saline of pH 7.8 was added to make a 15 to 20 ml. suspension. The cells were transferred to each of two 50 ml. flasks containing a Teflon® covered magnet. To one of the flasks were added 250 mg. (250,000 Armour units) of trypsin dissolved in 3 ml. of Sorenson's phosphate buffer (pH 7.1). The other flasks received 3 ml. of the buffer solution as a control. Both samples were incubated at 38°C. and magnetically mixed for five hours, after which time an additional 250 mg. of trypsin in 3 ml. of Sorenson's buffer were added to

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[2] This investigation was supported by grants from The Lancaster County Tuberculosis Association, Lincoln, Nebraska; The University of Nebraska Research Council; and Eli Lilly Company, Indianapolis, Indiana.

[3] Armour lyophilized crystalline trypsin (Tryptar) supplied by the Armour Laboratories, Chicago, Illinois.

TABLE 1

PROTOCOL FOR TRYPSIN TREATMENT OF MYCOBACTERIUM TUBERCULOSIS

A) Pooled 28-day cells

↓

B) Refrigerated 2–5 days

↓

C) Media

a) Petragnani and nutrient agar slants
b) Dubos liquid medium
c) Thioglycollate broth

C) Stain: Acid-fast, Gram

↓

D) Wash 3× with sterile phosphate buffered saline, pH 7.8

↓

E) Cells divided into two equal portions containing 1 ml. of packed cells each. Dispersed. Nitrogen determinations; plate counts on Dubos oleic-acid–albumin agar medium. Bring to volume of 15 to 20 ml. with saline diluent pH 7.8.

F) Add 250 mg. trypsin in 3 ml. Sorenson's phosphate buffer, pH 7.1	F) Add 3 ml. Sorenson's phosphate buffer only
↓	↓
G) Magnetically mixed 5 hours	G) Magnetically mixed 5 hours
↓	↓
H) Repeat F and continue magnetic mixing 5 hours	H) Repeat F and continue magnetic mixing 5 hours
↓	↓
I) Wash 3× with buffered saline, pH 7.1	I) Wash 3× with buffered saline, pH 7.1
↓	↓
J) Dispersed. Nitrogen determinations; plate counts on Dubos medium (unaltered cells) and nutrient agar plates (altered cells)	J) Dispersed. Nitrogen determinations; plate counts on Dubos medium (unaltered cells) and nutrient agar plates (altered cells)
↓	↓
K) Surface alteration studies	K) Surface alteration studies

the flask containing trypsin, and 3 ml. of diluent only to the control flask. Both samples were magnetically mixed for an additional five hours. Aliquots of the supernatant of trypsin and cell mixture were assayed at various time intervals for enzymatic activity. The substrate casein and acetic acid alcohol mixture were used to test the degree of hydrolysis which occurred during the incubation of the enzyme and substrate. The absence of the precipitate was indicative of maximal trypsin activity (3). The cells were then washed three times with buffered saline (pH 7.1), dispersed, and nitrogen determinations and Dubos and nutrient agar plates were prepared for bacterial counts. The cells were then stored at −5° C. until used. Storage time never exceeded twelve hours.

It was believed that surface modification could be demonstrated most satisfactorily by determining whether mercuric ions would penetrate more rapidly or be absorbed by the enzyme-treated tubercle bacilli. The trypsin- and nontrypsin-treated cells were suspended

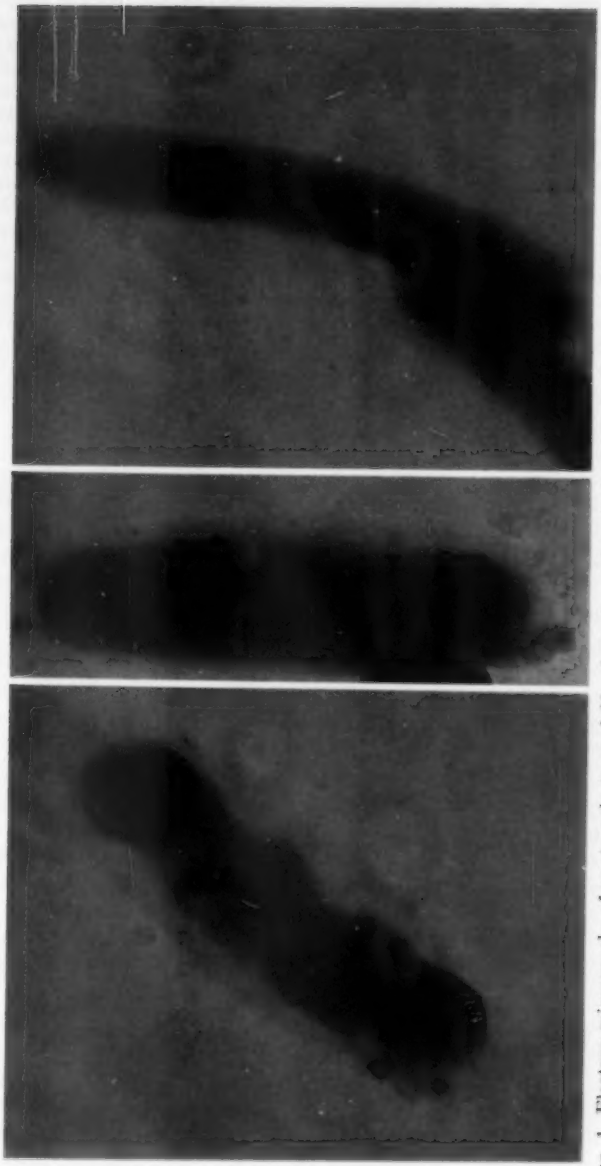

FIG. 1. Electron micrographs of nontrypsin-treated *Mycobacterium tuberculosis* exposed to mercuric chloride, washed, and treated with hydrogen sulfate.

in 0.01 M mercuric chloride (4) and shaken at room temperature for four minutes, then washed twice with neutral distilled water to remove the mercuric ions not combined with the cells. Bubbles of hydrogen sulfide were passed through the suspension of treated and nontreated cells after which the cells were again washed. The bacilli were then prepared for electron microscopy and observed for the presence of mercuric sulfide granules. Confirmation of these particles was evidenced by analysis of X-ray diffraction lines with regard to intensities and planar spacing (5). The treated cells were washed, dried at 110°C., pulverized in a mortar, and the powder was used for X-ray diffraction determinations.

For the preparation of supporting films on specimen holders for electron microscopy (6), the copper grids were washed with 0.1 per cent nitric acid and rinsed in distilled water several times and dried. A shallow circular dish was filled to overflowing with distilled water and a drop of collodion (2 per cent prepared in amyl acetate) was placed on the surface of the water. The radial spread rapidly, forming a circular patch. This first film was discarded, as it served to sweep the water surface free of dust. The grids were placed, smooth side down, on the second film. Within three minutes, the collodion film was removed by immersion of a glass slide forcing the film to fold back against the slide surface. The slide was then turned and lifted clear of the liquid, and excess water was blotted off and placed in the desiccator for several days. The bacterial suspension was placed on each grid with a capillary pipette after which the grids were again placed in the desiccator for several days before observations were made.

Observations

Representative electron photomicrographs of nontrypsin-treated *Mycobacterium tuberculosis* (H37Rv) exposed to the action of mercuric chloride and hydrogen sulfide are shown in figure 1. The relatively large distinct cell wall area, the darker appearing bipolar section of the cytoplasm, and the absence of mercuric sulfide granules within the cell are characteristic. With the treated cells (figure 2), however, similar observations revealed the presence of mercuric sulfide granules as many tiny dark precipitates, indicating absorption or penetration of the mercuric ions. Furthermore, while granules are uniformly distributed in the intact cell wall area, there appear to be more aggregates apparent on the cytoplasmic portions of the cell. Indirect evidence that this may be the case is supported by electron microscopy in that granules appear in sharp focus at one setting only, whereas, if the phenomenon were predominately surface adsorption, other precipitates would be expected to appear at different levels, as with the cell wall granules. The latter are observed in one of the preparations (figure 2*B*) wherein the cell wall has become separated from the cytoplasmic portion of the bacilli. These separated portions are readily discernible because of the presence of mercuric sulfide granules. Furthermore, occasionally spherical structures (bipolar aggregates) were observed scattered throughout the field, undoubtedly released by disruption of the tubercle bacilli (figure 2*B*).

With the concentration of trypsin employed, however, cell destruction generally did not occur. Direct evidence to support this was provided by observing plate counts before and after trypsin treatment. The results indicated no appreciable loss of bacilli and little loss of cellular nitrogen. Total cell numbers (Dubos and nutrient agar) of trypsin-treated, unaltered, altered, dispersed bacilli were equivalent to total cell numbers recorded (Dubos agar medium) prior to treatment.

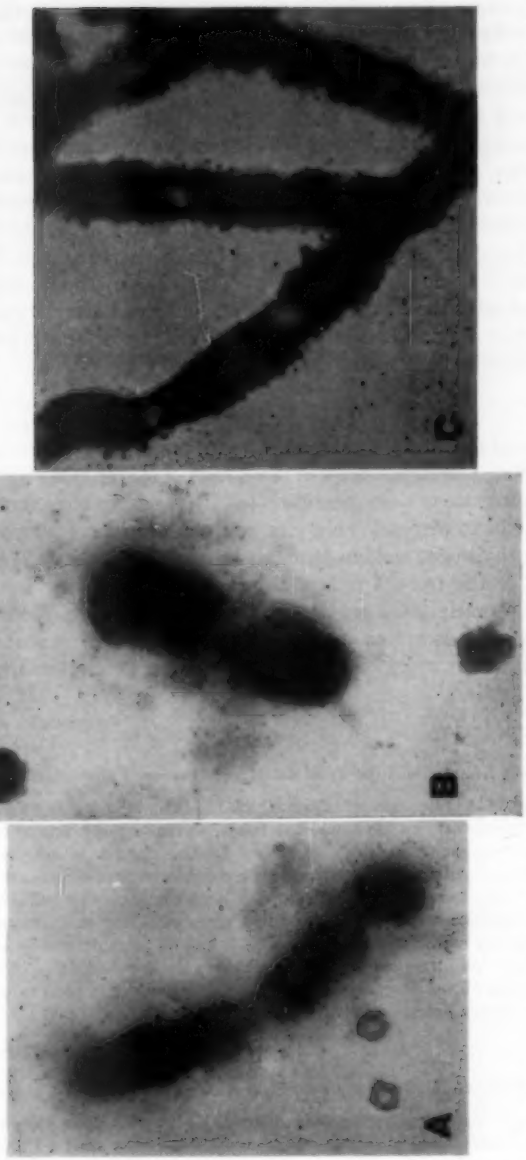

FIG. 2. Electron micrographs of trypsin-treated *Mycobacterium tuberculosis* exposed to mercuric chloride, washed, and treated with hydrogen sulfate.

Admittedly with this method, alteration of all bacilli with trypsin was impossible since the enzyme was unable to penetrate the few existing tightly clumped aggregates of cells. By dispersion prior to plating, such unaltered cells were detected in the same way as the nontreated cells. While the treated cells grew readily on nutrient agar within forty-eight hours (7), the nontreated bacilli repeatedly failed to exhibit such an alteration of growth. These observations, although not indicating what specific cell inclusions have a predilection for these mercuric cations, provided indirect evidence that enzyme treatment of these bacilli has modified the cell surface, facilitating permeation of these ions. Whether this surface modification is reflected in the decreased time interval required to reduce methylene blue or increases the vigor of oxidative attack of certain substrates remains to be determined. However, with the method outlined above, it has been noted that the stained preparation observed after trypsin treatment showed that this enzyme had effected single cell dispersement while the nontreated cells remained clumped.

Summary

Observations of the intrapleural application of trypsin (Tryptar®) in tuberculous empyemas initiated *in vitro* studies of the effects of this enzyme upon *M. tuberculosis*. Surface alterations were demonstrated by exposure of enzyme-treated and nontreated bacilli to mercuric chlorides and observation of precipitates formed after bubbling hydrogen sulfide through the cell suspensions. Electron-microscopic studies of the treated cells indicate that the mercuric ions observed as mercuric sulfide precipitates are distributed uniformly throughout the cell wall area, with possible adsorption onto the cytoplasmic surfaces without, however, aggregation on the external cell surface area. These precipitates were absent in the nontreated cells.

Sumario

Estudios de la Alteración Superficial del Mycobacterium Tuberculosis por Tripsina

Observaciones de la aplicación intrapleural del triptar a la eliminación y destrucción del *Mycobacterium tuberculosis* en los empiemas tuberculosos hicieron iniciar estudios *in vitro* de los efectos de dicha encima sobre el microbio. Distinguiéronse alteraciones superficiales por la exposición de bacilos tratados y no tratados con la encima a cloruros mercúricos y la observación de precipitados formados después de lanzar burbujas de sulfuro de hidrógeno a través de las suspensiones de células. Estudios ejecutados con el microscopio electrónico indican que los yones mercúricos observados en forma de precipitados de sulfuro de hidrógeno se distribuyen uniformemente por toda la zona de la pared de la célula, con posible adsorción en las superficies citoplásmicas, pero sin agregación a la zona externa de la superficie de la célula. Estos precipitados no existían en las células no tratadas.

Resume

Etude de l'altération superficielle du Mycobactérium tuberculosis par la trypsine

Les observations sur l'application intrapleurale de tryptar pour enlever et détruire le Mycobactérium tuberculosis dans les empyèmes tuberculeux sont à l'origine des études entreprises *in vitro* sur les effets de cet enzyme sur le bacille. Les modifications superficielles furent démontrées par l'exposition des bacilles traités ou non traités par l'enzyme au

chlorure de mercure et à l'observation des précipités formés après barbotage de sulfure d'hydrogène dans les suspensions cellulaires. Les études micro-électroniques des cellules traitées indiquent que les ions mercuriques observés à l'état de précipités sont répartis uniformément dans toute la paroi cellulaire et il se peut qu'il y ait également une adsorption sur les surfaces cytoplasmiques sans agrégat au niveau de la surface cellulaire externe. Ces précipités étaient absents dans les cellules non traitées.

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Case Reports

PULMONARY GEOTRICHOSIS

B. H. WEBSTER[1]

(Received for publication October 11, 1956)

During the past decade, there has been increased interest in fungal diseases. Reports in the medical literature show that fungal infections have been more frequent since the widespread use of antimicrobial therapy. Superinfection by saprophytic invaders may follow damage to tissue, lowered tissue resistance, suppression of the primary organisms, and ecologic disturbance. When the etiologic agents of infection are suppressed, the normal flora may disappear, and saprophytic bacteria or fungi may become pathogenic secondary invaders. Overgrowths of this type assume many forms and affect different systems.

Geotrichum candidum appears as a saprophyte in the mouth, bronchi, and gastrointestinal tract of many normal persons. Under certain conditions this agent becomes invasive and leads to a rare disease affecting the bronchi and lungs. Only a few well-documented cases of geotrichosis have been described. Superimposed infections with Geotrichum may result in exacerbation of pre-existing bronchiectasis and pulmonary tuberculosis. Frequently the simultaneous occurrence of *G. candidum* and *Klebsiella pneumoniae* suggests the possibility of a symbiosis between these two organisms.

There are no specific symptoms or physical findings of bronchopulmonary geotrichosis. Characteristically, the sputum is earthy-smelling and is a gelatinous mucoid type with suspended grayish flakes. The chest films are not diagnostic, but may simulate numerous other diseases, e.g., tuberculosis, bacterial pneumonias, other pulmonary mycoses, and carcinoma of the lung.

The diagnosis of pulmonary geotrichosis depends upon repeated isolations of the etiologic agent from fresh sputum or bronchoscopic aspirates, repeated cultures, biopsy, and the elimination of other diseases. The Geotrichum intradermal test may indicate present or past disease.

Case Report

W. B. (March 24, 1956), a 46-year-old married white truck driver, was well until three weeks before admission to the hospital, when he arose with a fever of 105°F. and had two chills. He was treated with tetracycline (250 mg. every six hours for three days) for influenza and seemed to recover. Twenty-four hours before admission he had recurrence of fever and chills and began to cough constantly, producing a thick, slimy, rusty sputum with an earthy smell. Immediately following admission, he coughed convulsively and noted several mouthfuls of bright blood.

Past history was nonrevealing except for a routine normal chest plate three months previously. Family history was negative for tuberculosis, diabetes, or cancer.

Physical examination revealed a well-developed, well-nourished, acutely ill man with marked dyspnea. The temperature was 105.4°F., pulse 120, and the respirations 28 per minute. The pharynx was infected and the tonsils were enlarged. There was a respiratory lag over the left upper chest anteriorly. Over the left upper and midlung fields there was dullness to percussion and, on auscultation, many fine and coarse rales were noted and the breath sounds were decreased. The remainder of the examination was negative.

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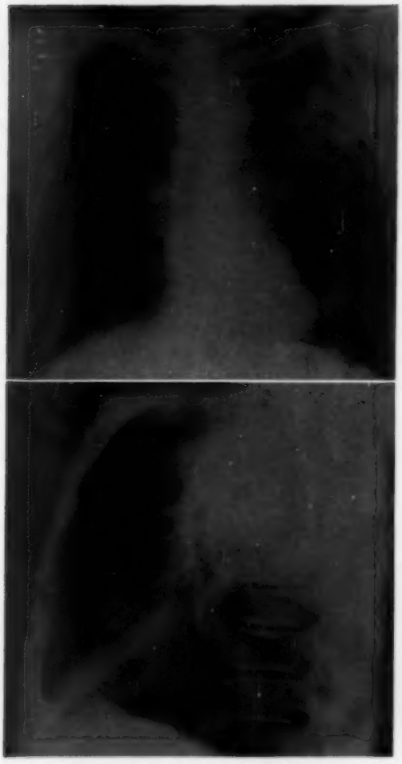

FIG. 1 (Upper). Chest roentgenogram (March 24, 1956), denoting infiltration and cavity of left upper lobe.

FIG. 2 (Lower). Lateral chest film (March 24, 1956), showing cavity and infiltration in the inferior portion of the posterior apical segment of left upper lobe.

A roentgenogram of the chest, postero-anterior and lateral (figures 1, 2) showed infiltration and a large thin-walled cavity in the inferior portion of the posterior apical segment of the left lower lobe. The remainder of the lungs was clear.

Laboratory examination revealed packed cell volume of 42 per cent; hemoglobin, 14.0 gm.; leukocyte count, 24,600 per cu. mm.; and a differential of 88 polymorphonuclear leukocytes, 7 lymphocytes, and 5 monocytes. The erythrocyte sedimentation rate was 26 mm. A serologic test for syphilis and the values for nonprotein nitrogen, fasting blood sugar, and a urinalysis were normal. A series of 12 Ziehl-Neelsen sputum smears were negative for acid-fast bacilli. Three cultures of sputum and gastric washings for *Mycobacterium tuberculosis* were negative. Culture on Sabouraud's medium grew typical *Geotrichum candidum* (figures 3, 4). Skin tests for Brucella, PPD (double-strength), histoplasmin, and coccidioidin were negative. Routine agglutinations were negative. Blood culture revealed *G. candidum* on two occasions. Culture of urine was sterile. Stool culture was negative for enteric organisms, but positive for *G. candidum*. Bronchoscopic examination recovered a gelatinous exudate which revealed the same laboratory findings.

The patient was placed on tetracycline for one week, then oral and intravenous potas-

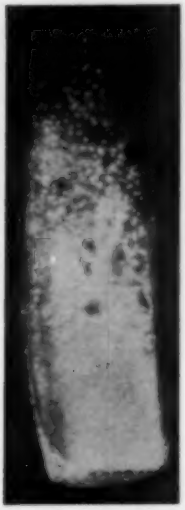

FIG. 3. Culture of *G. candidum* on Sabouraud's glucose agar.

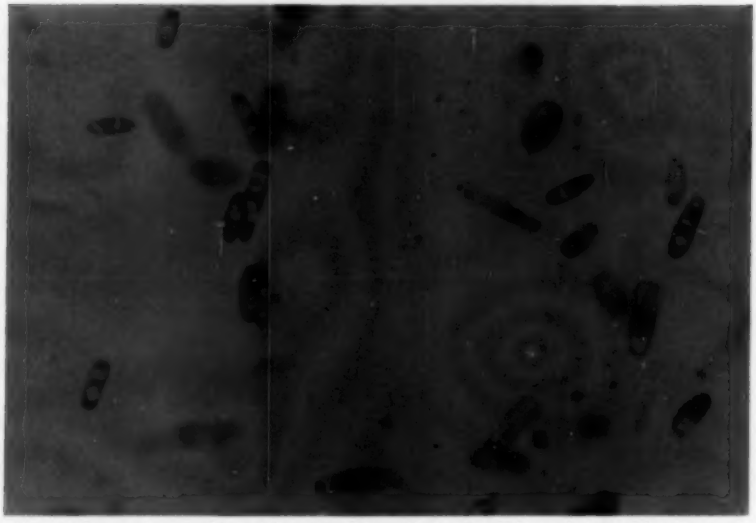

FIG. 4. Smear from culture shown in figure 3, showing *G. candidum* arthrospores.

sium iodide, bed rest, vitamins, and high-vitamin, high-caloric diet, with progressive clearing of the exudate and gradual closure of the cavity. After six months the chest film showed only peribronchial infiltration, but the sputum cultures are still positive for *G. candidum*. Blood culture has become negative. Six cultures for *M. tuberculosis* and two guinea pig inoculations have been negative. PPD (double-strength), coccidioidin, and histoplasmin tests remain negative. The Geotrichum intradermal test is strongly positive.

Comment

The patient herein presented represents an explosive onset of high fever, chills, hemoptysis, and roentgenographic findings of a thin-walled cavity and infiltration of the left lower lobe of the lung. *G. candidum* was isolated from the sputum by culture repeatedly and also from gastric washings and bronchoscopic aspirates. Tuberculin, histoplasmin, and coccidioidin tests were negative repeatedly. Two guinea pig inoculations were negative. The Geotrichum intradermal test was strongly positive.

The patient received 12 tablets of 250 mg. of tetracycline at the beginning of the illness. The infection appeared to be a primary one, but the question of previous alteration by the antimicrobial drug and the development of mycotic superinfection arises. This point in etiology occurs in this day of widespread use of antimicrobial drugs.

The problem evolves around the point that it is difficult to establish the fact that a particular patient is actually infected with an otherwise nonpathogenic fungus. From endogenous foci in man, *Geotrichum candidum* becomes pathogenic as a primary or secondary invader. Pulmonary tuberculosis, lung abscess, and neoplastic disease have been excluded by appropiate means.

The prognosis of geotrichosis is usually good, but grave sequellae may ensue. Spontaneous cures may occur. Such serious pulmonary findings as were observed in this patient are rare, but may become more frequent as a high index of suspicion as geotrichosis develops.

Lastly, this case illustrates the rare occurrence of Geotrichum septicemia. Only two such reports have been gleaned from the literature.

Satisfactory response to intravenous and oral iodides has led to progressive clearing of the infiltration and disappearance of the cavity. This is the present treatment of choice.

Summary

A case report of a 46-year-old white man with pulmonary geotrichosis is presented. Roentgenograms of the lung revealed a thin-walled cavity and infiltration of the left lower lobe. There was a concurrent Geotrichum septicemia. Treatment with oral and intravenous iodides has been effective.

Sumario

Geotricosis Pulmonar: Presentación de Un Caso con Septicemia

Discútese la geotricosis pulmonar.

Preséntase la historia clínica de un sujeto blanco de 46 años de edad que tenía geotricosis pulmonar. Las radiografías del pulmón revelaron una caverna de paredes delgadas e infiltración del lóbulo izquierdo. Había una septicemia por *Geotrichum* concomitante. El tratamiento oral e intravenoso con yoduros ha sido eficaz.

Resume

Géotrichose pulmonaire: Observation d'un cas avec septicémie

La géotrichose pulmonaire est commentée.

Observation d'un cas chez un homme de race blanche âgé de 46 ans. Les radiogrammes du poumon révélèrent la présence d'une excavation à paroi mince et l'infiltration du lobe gauche. Il y avait en même temps une septicémie à *Goetrichum*. Le traitement par les iodures par voie buccale et intraveineuse s'est montré efficace.

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ROENTGENOTHERAPY OF THE MIDDLE LOBE SYNDROME IN CHILDREN[1, 2]

Report of Two Cases

H. B. HATCH, JR., AND BUELL C. BUCHTEL

(Received for publication October 26, 1956)

Since the initial description of the middle lobe syndrome by Graham, Burford, and Mayer (1) in 1948, an increasing number of articles concerning this now accepted clinical entity have appeared in the literature. Most reported cases have been in adults, and the usually recommended therapy has been surgical removal of the diseased lobe. Two children with the middle lobe syndrome of short duration recently seen by the present writers were successfully treated by *radiation*. It is the purpose of this paper to report these cases and to advocate roentgenotherapy in similar cases. A number of other patients treated in this way have not been followed long enough to be included in this report.

Report of Cases

Case 1. I. J. B., a white boy, 7 years old, was admitted to the Ochsner Foundation Hospital on March 30, 1954. Eleven days prior to admission he had had a paroxysmal attack of coughing which lasted as long as four hours, then decreased in severity. Paroxysms would occur two to three times within a period of twenty-four hours. Occasionally, actual whooping would occur. During this time the temperature ranged between 100° and 101° F., but the patient had no chills. At the onset of the illness the sputum was mucopurulent, but just prior to admission the cough became nonproductive. There was no history of aspiration or thoracic pain.

The only pertinent positive finding on physical examination was the presence of decreased breath sounds over the lower anterior aspect of the right lung. The hemogram was normal. Urinalysis and serologic tests for syphilis yielded negative results. The sputum was negative for acid-fast bacilli, and no predominant organism was isolated. The result of the tuberculin skin reaction was negative and has remained so to date.

Bronchoscopy disclosed frank pus in the right main stem bronchus and left lower lobe bronchus. The middle lobe orifice was extremely edematous and inflamed.

A roentgenogram of the chest obtained approximately one year prior to admission showed no abnormalities. A roentgenogram of the chest obtained on March 30, 1954, revealed a triangular opacity extending laterally and downward from the lower pole of the right hilum (figure 1). The right lateral roentgenogram showed a triangular opacity in the region of the right middle lobe indicating a reduction in volume of the lobe (figure 1).

A bronchogram revealed normal upper and lower lobes (figure 2). There was a point of narrowing at the bifurcation of the middle and lower lobes and only a short portion of the middle lobe bronchus was visualized. The axillary division of the upper lobe fanned inferiorly.

The patient was not given antimicrobial therapy. From April 5 through April 9, 1954, he received 75 roentgens daily as outlined. A roentgenogram obtained on April 17, 1954, showed definite clearing of the density, and results of subsequent films have been normal (figure 3). The patient has remained asymptomatic to date.

[1] From the Section on Chest Diseases and Department of Radiology, Ochsner Clinic, New Orleans, Louisiana.

[2] Presented before the Twelfth Annual Meeting of the American College of Chest Physicians (Southern Chapter), in Houston, Texas, November 13-14, 1955.

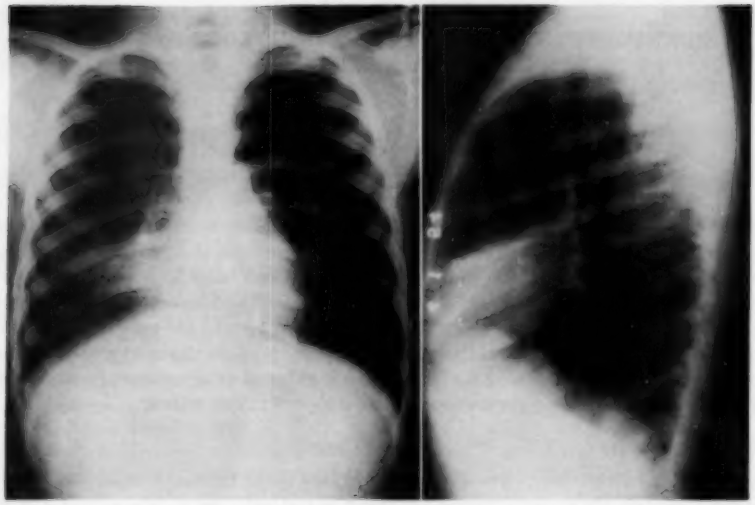

FIG. 1. *Case 1.* Postero-anterior and lateral roentgenograms of middle lobe syndrome.

Case 2. J. A., a white boy, 10 years old, was admitted to the Ochsner Foundation Hospital on August 7, 1955. Three days before, he complained of cramping pain in the right upper abdominal quadrant associated with one episode of vomiting. There was no diarrhea. The temperature ranged from 102° to 104° F. and he had no chills. He had had a slight, hacking, nonproductive cough during the illness. There was no history suggestive of pleuritic pain or aspiration.

The temperature on admission was 103° F. There were a few petechiae on the hard palate, a grade I apical systolic murmur, and slight tenderness in the right upper abdominal quadrant. The lungs were clear.

The hemogram was normal. Results of urinalysis and tests of liver function were also normal. No predominant organism could be recovered in the sputum. The tuberculin reaction was negative and has remained so. A roentgenogram of the chest obtained on August 8, 1955, showed typical evidence of the middle lobe syndrome (figure 4).

The patient did not receive antimicrobial therapy. On August 18, 1955, roentgenotherapy was begun, as in the preceding case. A roentgenogram of the chest obtained on August 25, 1955, showed disappearance of the lesion (figure 5). The results of subsequent films have been normal, and the child has remained asymptomatic.

COMMENT

The anatomic, pathogenic, and clinical aspects of the middle lobe syndrome will be considered only briefly here, as they have been thoroughly discussed by others (2, 3). The middle lobe syndrome is a suppurative process initiated by obstruction of the middle lobe bronchus as a result of lobar hilar lymphadenopathy, originally described as nontuberculous. This obstruction results in bronchostenosis, pneumonia, and finally collapse of the lobe. The eventual fate of the lobe depends upon the duration of the obstruction and the virulence of the infection. It may re-expand and return to normal; it may re-expand but contain residual bronchiectasis; or it may remain collapsed and become functionless (figure 6) (3). The ideal therapeutic result is obviously complete expansion with a normal

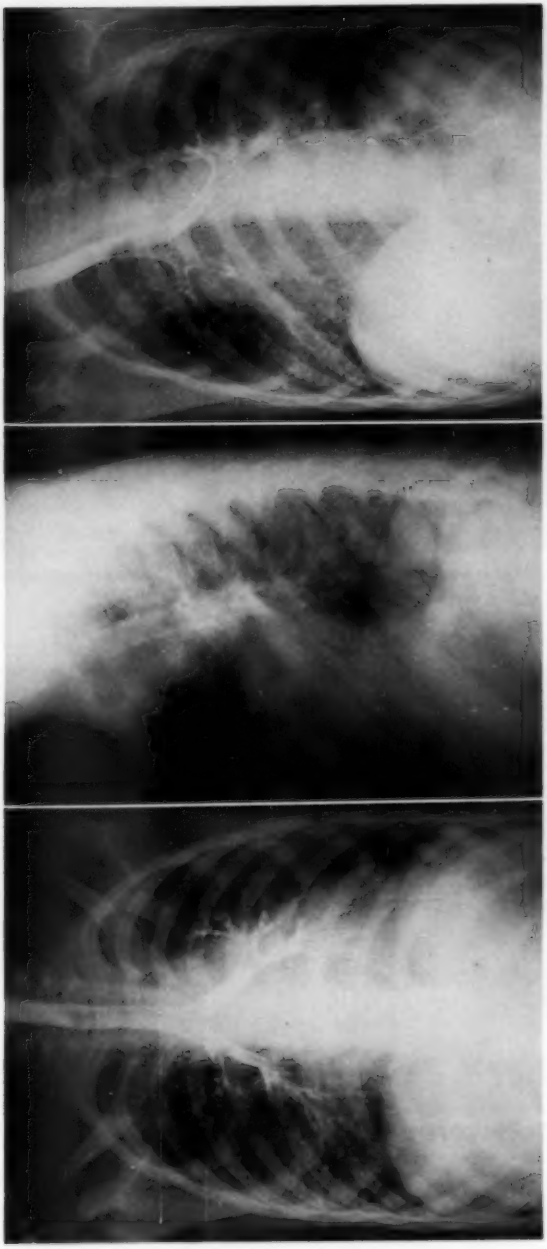

FIG. 2. *Case 1.* Bronchograms (postero-anterior, lateral, and oblique views) demonstrating obstruction of middle lobe bronchus.

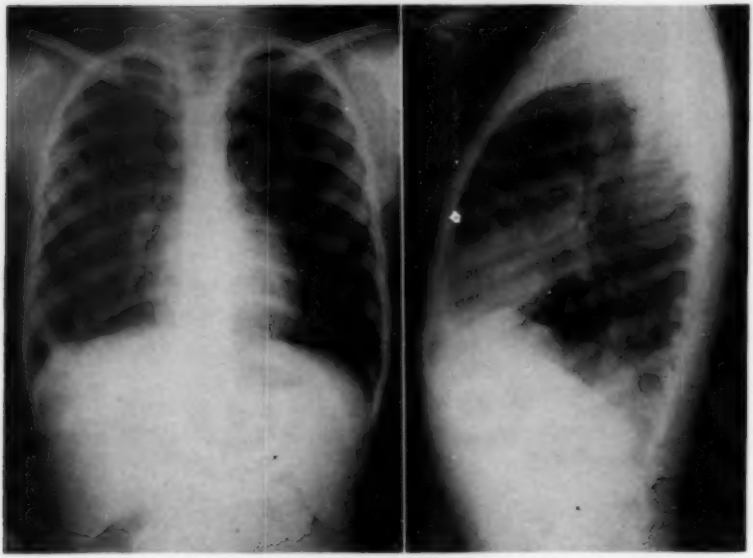

FIG. 3. *Case 1.* Postero-anterior and lateral roentgenograms following radiation showing definite clearing of the density.

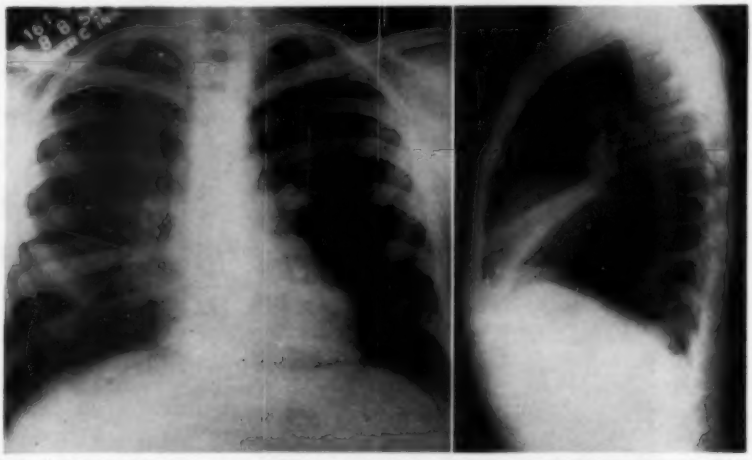

FIG. 4. *Case 2.* Postero-anterior and lateral roentgenograms showing typical evidence of middle lobe syndrome.

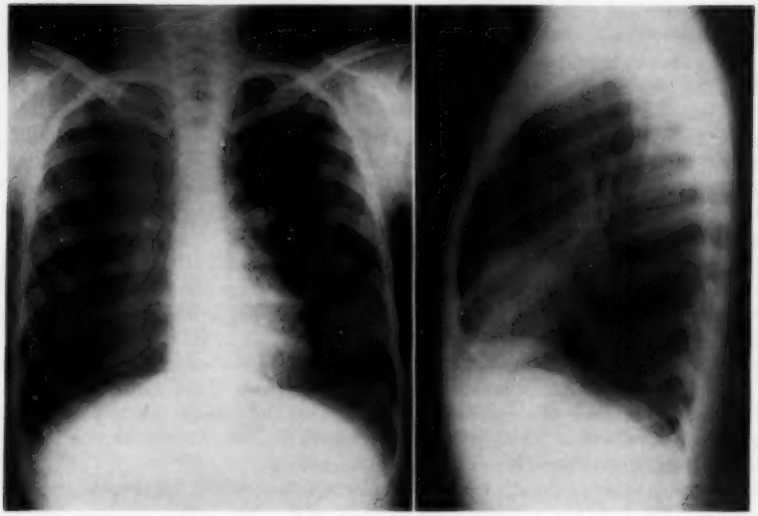

FIG. 5. *Case 2.* Postero-anterior and lateral roentgenograms following radiation showing definite clearing of the density.

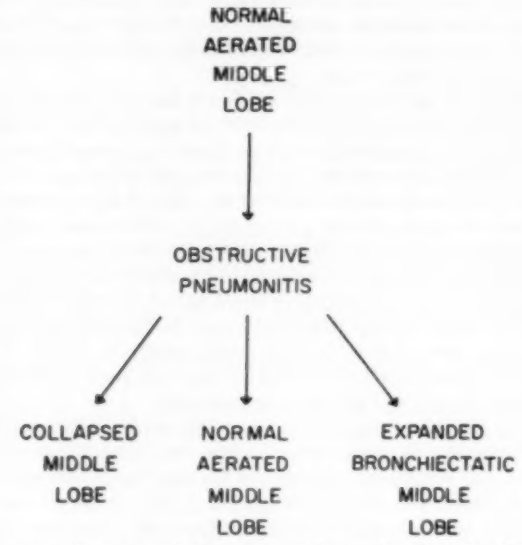

FIG. 6. Diagrammatic representation of possible results of obstruction to middle lobe bronchus.

bronchial tree and pulmonary parenchyma capable of carrying on the primary function of pulmonary tissue, that is, exchange of oxygen and carbon dioxide.

There are two important anatomic features in the pathogenesis of middle lobe collapse. First, the lymphatic drainage of the middle lobe and part of the lower lobe into lymph nodes surrounding the middle lobe bronchus, as described by Brock and associates (4), makes the middle lobe bronchus particularly susceptible to compression of its lumen. Second, there are peculiarities of the middle lobe bronchus itself, such as its length, its pliability, and the angle of divergence, which add to the predisposition to collapse. With these factors in mind, it is not difficult to see that inflammatory disease involving the middle lobe, lower lobe, or both, can produce this syndrome.

The syndrome may cause no symptoms at all, being detected only on routine roentgenographic examination of the chest. More often, however, symptoms such as cough, varying amounts of sputum, fever, thoracic pain, and (occasionally) hemoptysis, will be present. Children may have only systemic manifestations (malaise, irritability, myalgia) or, as in one of the cases herein presented, the symptoms may be predominately gastrointestinal.

The physical examination may be helpful in the diagnosis of the middle lobe syndrome. However, the presence of abnormalities on percussion and auscultation located anteriorly in the inferior aspect of the right hemithorax would depend upon the stage of the pathologic process. Hence, there is no one pathognomonic sign.

Three important diagnostic tools are available, roentgenography, bronchography, and bronchoscopy. A postero-anterior roentgenogram of the chest will show a well-demarcated density in the medial portion of the right lower pulmonary field and depression of the horizontal fissure. A lateral roentgenogram will show depression of the horizontal fissure and elevation of the oblique fissure with increased density between the two. Occasionally, hilar lymphadenopathy may be seen. Bronchography will confirm the presence of obstruction and will often demonstrate the overexpansion of the right upper lobe. Bronchoscopy will reveal inflammation and edema in the region of the middle lobe orifice, and occasionally it will show extrinsic compression.

The usual therapy of the middle lobe syndrome has been lobectomy. The present writers have not been impressed with the effect of antimicrobial therapy, used either alone or as an adjunct to symptomatic therapy. Therefore, there is a definite need for an effective, uncomplicated, and easily accomplished method of therapy.

The rationale of radiation in the treatment of the middle lobe syndrome is based on two peculiarities of the disease mentioned previously: (*1*) inflammatory enlargement of the peribronchial lymph nodes, and (*2*) limitation of these nodes to the zone of the bifurcation of the right middle lobe bronchus. So radiosensitive are these nodes that it is possible to employ small repeated doses of roentgen ray which are adequate to control the pathologic process but will not cause damage to normal tissues in the path of the irradiation. The location of the nodes makes it possible to use small coned fields directly over the area. In children, the small anteroposterior diameter of the chest permits administration of an adequate depth dose in terms of the small air dose used.

It has long been recognized that small repeated doses of roentgen-ray irradiation produce a favorable response in certain inflammatory conditions. Furunculosis, nonspecific adenitis, parotitis, and lymphoid hyperplasia of the nasopharynx are some of the diseases that have been successfully treated in this manner. Lymphocytes are vulnerable to small doses of irradiation, and reduction in volume of lymphoid tissue may be observed in a few days following institution of roentgenotherapy.

Precise centering of the portals allows the use of small-field size. The present writers

have employed portals no larger than 7 by 7 cm., directed anteriorly, posteriorly, or both, over the thorax.

The physical factors employed in the treatment are: 250 kilovolts, 15 milliamperes; half value layer 1.5 mm. copper, 50 cm. focal-skin distance. Treatments of 75 roentgens per portal are given daily for six consecutive days for a total air dose of 450 roentgens. In the 2 cases reported, the entire irradiation was administered through only one portal in order to simplify treatment. No cutaneous reaction was observed in the treated area and there were no untoward systemic effects.

It is believed that roentgenotherapy is the most efficacious therapy, since it removes the major factor responsible for the development of the disease; that is, the mechanical obstruction of the middle lobe bronchus by the enlarged peribronchial lymph nodes. However, this therapy is most effective when instituted during the early phases of the development of the syndrome. The longer treatment is delayed, the greater the possibility of irreversible changes within the lobe itself.

Summary

Roentgen-ray irradiation is offered as a new effective method of treating particularly the early stages of the middle lobe syndrome in children. Two cases demonstrating the successful results of such therapy are presented.

Sumario

Roentgenoterapia del Síndrome del Lóbulo Medio del Pulmón en los Niños: Presentación de Dos Casos

Se ofrece la irradiación roentgen como nuevo método eficaz para tratar en particular las etapas incipientes del síndrome del lóbulo pulmonar medio en los ninos. Preséntanse 2 casos que demuestran el resultado feliz de esa terapéutica.

Resume

Radiothérapie dans le Syndrome du lobe moyen chez l'enfant: Observation de deux cas

La radiothérapie est présentée comme une nouvelle méthode efficace pour le traitement du syndrome du lobe moyen chez l'enfant, en particulier au cours du stade initial. Deux cas démontrant les résultats satisfaisants de cette thérapeutique sont présentés.

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(1) Graham, E. A., Burford, T. H., and Mayer, J. H.: Middle lobe syndrome, Postgrad. Med., 1948, *4*, 29.

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(4) Brock, R. C., Cann, R. J., and Dickinson, J. R.: Tuberculous mediastinal lymphadenitis in childhood: Secondary effects on lungs, Guy's Hosp. Rep., 1937, *87*, 295.

Notes

THE RELATIONSHIP BETWEEN DRUG RESISTANCE AND THE GROWTH PHASE OF MYCOBACTERIUM AVIUM[1]

It is known that the frequency with which mutants appear within a bacterial population varies with the growth phase of the organism in question.[2-4] With respect to drug-resistant mutants, Tsukamura[5] reported in 1951 that young nonacid-fast organisms within a strain of *Mycobacterium avium* were more resistant to streptomycin than older acid-fast cells. More recently, Van Orden and Kirchheimer[6] described similar observations made with a strain of *Mycobacterium smegmatis*, indicating that the organisms were more resistant to streptomycin during the early stages of the growth cycle than during later stages.

The present communication presents results obtained in a study designed to evaluate systematically the relationship between the growth phases of a strain of *Mycobacterium avium* and the frequency with which naturally occurring streptomycin- and isoniazid-resistant mutants occur within that strain.

The Jucho strain of *Mycobacterium avium* was used throughout the study. The strain was maintained in a modified Sauton medium,[7] by serial transfer of seven-day-old cultures. In all instances, care was taken to use for transfer a small inoculum containing not more than several hundred culturable cell units.

Seven-day-old stock cultures, as prepared above, were centrifuged and resuspended in saline to yield a culture suspension containing approximately 500 culturable cell units per ml. Flasks containing 50 ml. of modified Sauton medium were then inoculated with 1 ml. of cell suspension, the resultant culture thus containing approximately 10 culturable cell units per ml. of medium. Cultures were incubated at 37°C. for a period of fourteen days.

At intervals throughout the incubation period, portions of each culture were centrifuged at 3,000 r.p.m. for twenty minutes, the resultant supernatant was discarded, and the sedimented cells were shaken with glass beads for ten minutes. Fifty milliliters of saline were then added to the cell mass, the suspension was mixed, and the number of milligrams, wet weight, of bacterial cells per milliliter of suspension was determined.[8] The cell suspension

[1] Because of space limitation, it was necessary to condense the original manuscript submitted by Drs. Michio Tsukamura and Koji Miura. Accordingly, with their permission, the manuscript was turned over to Dr. Gladys L. Hobby of Chas. Pfizer & Co., Inc., for reorganization and condensation. The authors of the manuscript and the Editor of the REVIEW wish to express their appreciation to Dr. Hobby for her very considerable contribution to the publication of this report.

[2] LURIA, S. E.: Bact. Rev., 1947, *11*, 1.

[3] CATCHESIDE, D. G.: The Genetics of Microorganisms, Pitman Publishers, London, 1949.

[4] BRAUN, W.: Bacterial Genetics, W. B. Saunders Company, Philadelphia, 1953.

[5] TSUKAMURA, M.: Kekkaku, 1951, *26*, 263.

[6] VAN ORDEN, L. S., AND KIRCHHEIMER, W. F.: Am. Rev. Tuberc., 1955, *71*, 743.

[7] The modified Sauton medium used herein contained 50 ml. of glycerin, 2.0 gm. of citric acid, 0.5 gm. of magnesium sulfate, 0.5 gm. of dibasic potassium phosphate, 0.05 gm. of ferric ammonium citrate, 4.0 gm. of sodium glutamate, 950 ml. of distilled water. The pH was adjusted to 7.2 with 10 per cent sodium hydroxide, and the resultant medium was dispensed in 50 ml. amounts in 200 ml. Ehrlenmeyer flasks and autoclaved at 115°C. for thirty minutes.

[8] The number of milligrams, wet weight, of cells per milliliter of suspension was determined

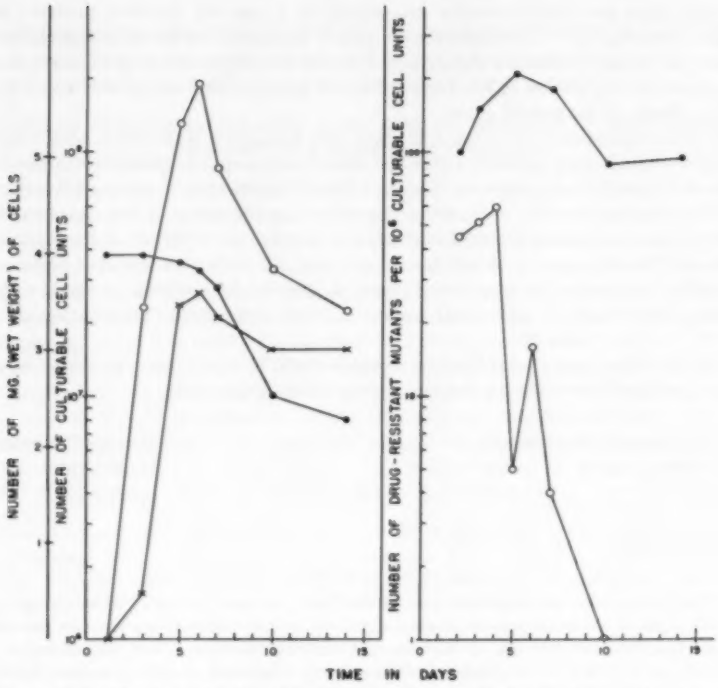
NUMBER OF MG. (WET WEIGHT) OF CELLS
NUMBER OF CULTURABLE CELL UNITS
NUMBER OF DRUG-RESISTANT MUTANTS PER 10⁶ CULTURABLE CELL UNITS
TIME IN DAYS

FIG. 1. Left: Number of culturable cell units: ○——○ observed, per ml. of medium ●——● estimated, per mg. (wet weight) of cells. Number of mg. (weight of cells): ×——× estimated, per ml. of medium. Right: ○——○ number of mutants resistant to 10 γ of streptomycin, and ●——● number of mutants resistant to 10 γ of isoniazid per 10^6 culturable cell units.

was then recentrifuged, the supernatant was again discarded, and the cells were vigorously shaken with glass beads for another ten-minute period. Finally a sufficient volume of saline was added to give a concentration equivalent to approximately 1 mg., wet weight, of bacterial cells per ml., and the resultant suspension was allowed to stand for thirty minutes so that any residual large clumps might settle out. The number of culturable cell units per milliliter of suspension was then determined in the conventional manner.[9] From this value, it was possible to calculate the number of culturable cell units per milliliter of culture medium, and to estimate both the number of culturable cell units per milligram of cells (wet weight) and the wet weight of cells per milliliter of medium.

The number of streptomycin- and isoniazid-resistant mutants naturally occurring in

by measurement of its optical density using a Leitz Rouy-Photometer, and by comparison of readings with a previously prepared standard curve.

[9] One-tenth milliliter of each of three dilutions (10^{-4}, 10^{-5}, 10^{-6}) of the cell suspension was inoculated with a roller onto each of ten plates containing the previously described Sauton medium (modified) to which had been added 3 per cent agar. Plates were incubated at 37°C. for four days, and the number of colonies was then counted in the usual manner.

the population was simultaneously determined by a one-step selection process[10] using media containing 10 γ of streptomycin[11] and of isoniazid.[12] Observations made in this manner at repeated intervals throughout a period of fourteen days' incubation showed the proportion of resistant to total culturable cell units per milliliter of suspension during various phases of the growth cycle.

In those experiments conducted to date, the frequency with which streptomycin-resistant mutants were detected within the otherwise susceptible population of the Jucho strain of *Mycobacterium avium* was highest (40 to 60 resistant mutants per 10^6 culturable cell units) during the early part of the logarithmic growth phase of the organism. A decrease in the incidence of resistant mutants was noted toward the end of the logarithmic phase, and the frequency with which such resistant cell units were observed became progressively less during the stationary phase of growth. In contrast, isoniazid-resistant mutants were observed with equal frequency at all observation points throughout the growth cycle (*see* figure 1).

From the data presented it may be assumed that the mechanisms by which streptomycin- and isoniazid-resistant mutants appear differ significantly.

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Koji Miura

March 13, 1957

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[10] The selection of resistant cells was accomplished by inoculation of 0.1 ml. of appropriate dilutions of the bacterial suspension onto each of two series of plates, one containing 10 γ of streptomycin per ml. of Sauton agar modified and the other containing 10 γ of isoniazid per ml. of this solid medium. Plates were incubated at 37°C. for four days, and the number of colonies per ml. of suspension capable of growing in the presence of the antimicrobial agents was determined.

[11] Sterile dihydrostreptomycin sulfate, manufactured by Kyowa Fermentation Industry.

[12] Isoniazid, manufactured by Tanabe Pharmacal Co., and sterilized by Seitz filtration of solutions.

[13] The writers wish to express their appreciation to Dr. Rokuro Katsunuma, Director of the Obuso National Sanatorium; and to Prof. Susumu Hibino, Medical School of Nagoya, for their advice and help in the conduct of this study.

THE DECREASE OF MUTATION RATE TO STREPTOMYCIN RESISTANCE IN SULFATHIAZOLE-RESISTANT MYCOBACTERIUM AVIUM PRODUCED BY THE PRESENCE OF SULFATHIAZOLE

A Specific Effect of Sulfathiazole in Reducing the Emergence of Streptomycin Resistance by a Nonselective Mechanism

The writer[1] reported on the effect of sulfathiazole in preventing the emergence of streptomycin resistance in a sulfathiazole-resistant strain of *Mycobacterium avium*. When tested by the serial transfer method, 10 γ of sulfathiazole prevented almost completely the emergence *in vitro* of streptomycin resistance in the strain, whereas the addition of the drug at the same concentration did not prevent the emergence of isoniazid resistance. The concentration of sulfathiazole used was never inhibitory for its growth. The purpose of the present paper is to clarify the mechanism of these phenomena.

In another report concurrently presented, the writer[2] has reported that the mutation rate to streptomycin resistance in the strain is smaller than that in the original susceptible strain of *Mycobacterium avium*. In the present study, the author reports that the decrease of mutation rate to streptomycin resistance in the strain is produced by the addition of sulfathiazole to culture media. These results appear to interpret very well a number of phenomena previously reported from this laboratory.[1,3]

The strain used is a sulfathiazole-resistant strain of *Mycobacterium avium*, strain Jucho. Its morphologic characteristics have previously been described.[3] The strain was isolated from the original parent strain by transferring it repeatedly by increasing the amount of the drug. After forty steps of selection by the drug, a strain resistant to 80 γ of sulfathiazole in Sauton medium was obtained, whereas the original parent strain was resistant to 0.25 γ of sulfathiazole per ml. of medium. The characteristic drug resistance of the strain was maintained through twenty transfers on media containing no drug. Thus, it was considered to be a stable heritable property. Almost all of the individual cells of the sulfathiazole-resistant strain were able to grow on Sauton agar plates containing 10 γ of sulfathiazole per ml. of medium, and more than ninety per cent of individual cells of its population were able to grow on Sauton agar plates containing 20 γ of the drug per ml. of medium. In contrast, almost all of the individual cells of the original parent strain were completely inhibited by the presence of sulfathiazole at a concentration of 0.5 γ per ml. of medium.

Sauton agar plates and Sauton medium were used in the study, in which sodium glutamate was substituted for asparagin. The pH of the medium was adjusted to pH 7.0 after autoclaving at 115°C. for thirty minutes. Sauton agar medium was dissolved in a water bath, and each 20 ml. of the medium were poured into a Petri dish, 9 cm. in diameter. Dihydrostreptomycin sulfate,[4] isoniazid,[5] and sulfathiazole[6] were used. These drugs were added to the medium in solutions prepared immediately before use.

Determination of the numbers of mutants resistant to streptomycin and to isoniazid were performed as follows. Cultures were collected and homogenized by shaking with glass beads for ten minutes and suspending in saline. A series of tenfold dilutions of the suspension were prepared. Samples of each 0.1 ml. of the original suspension were planted onto Sauton agar plates containing 10 γ of streptomycin per ml. of medium; samples of

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[1] Tsukamura, M.: J. Antibiot., *Series A*, 1956, *9*, 16.
[2] Tsukamura, M.: Am. Rev. Tuberc., 1957, *76*, 298.
[3] Tsukamura, M.: Med. & Biol., 1955, *35*, 123.
[4] Manufactured by Kyowa Co.
[5] Manufactured by Tanabe Co.
[6] Manufactured by Takeda Co.

TABLE 1

STREPTOMYCIN-RESISTANT MUTANTS PER 10^{10} VIABLE CELLS OCCURRING IN CULTURES GROWN ON SAUTON MEDIUM CONTAINING NO DRUG (MEDIUM O) AND IN CULTURES GROWN ON SAUTON MEDIUM CONTAINING 20 γ OF SULFATHIAZOLE PER ML. OF MEDIUM (MEDIUM ST)

Medium	Age of Cultures	Average Number of Viable Cells Planted for Each Plate	Average Number of Mutants per Each Plate	Mutants per 10^{10} Viable Cells
	days			
O	6	149.6×10^6	12.2	748.0
O	8	497.5×10^6	2.5	50.2
O	10	193.8×10^6	2.2	114.0
O	13	189.0×10^6	1.8	95.4
ST	6	271.5×10^6	2.4	88.8
ST	8	226.6×10^6	0.2	8.8
ST	10	280.0×10^6	0.8	28.6
ST	13	214.0×10^6	0.4	18.7

TABLE 2

ISONIAZID-RESISTANT MUTANTS PER 10^8 VIABLE CELLS OCCURRING IN CULTURES GROWN ON SAUTON MEDIUM CONTAINING NO DRUG (MEDIUM O) AND IN CULTURES GROWN ON SAUTON MEDIUM CONTAINING 20 γ OF SULFATHIAZOLE PER ML. OF MEDIUM (MEDIUM ST)

Medium	Age of Cultures	Average Number of Viable Cells Planted for Each Plate	Average Number of Mutants per Each Plate	Mutants per 10^8 Viable Cells
	days			
O	6	149.6×10^6	256.0	17.10
O	8	497.5×10^6	111.4	2.24
O	10	345.6×10^6	168.4	4.86
O	13	189.0×10^6	137.2	7.25
ST	6	271.5×10^6	137.0	5.05
ST	8	226.6×10^6	20.2	0.89
ST	10	232.4×10^6	92.4	3.96
ST	13	214.0×10^6	303.6	14.20

0.1 ml. of 10^{-2} dilution were planted onto Sauton agar plates containing 10 γ of isoniazid per ml. of medium; and samples of 0.1 ml. of 10^{-6} dilution were planted onto Sauton agar plates containing no drug. The numbers of viable colonies on these plates were determined after a four-day incubation period, and the number of mutants resistant to 10 γ of streptomycin and the number of mutants resistant to 10 γ of isoniazid per viable cells were calculated. Five plates were used for determination of the number of viable cells and the number of isoniazid-resistant mutants, and ten plates were used for determination of the number of streptomycin-resistant mutants. Therefore, twenty plates were used for each experiment. All cultures were incubated at 37°C. throughout the study.

Experiment 1: A series of 50 ml. of Sauton medium containing no drug and containing 20 γ of sulfathiazole per ml. of medium in 200 ml. Erlenmeyer flasks were inoculated with approximately several hundred viable cells and incubated at 37°.C After a five-day incu-

TABLE 3

STREPTOMYCIN-RESISTANT MUTANTS PER 10^{10} VIABLE CELLS OCCURRING IN CULTURES GROWN ON SAUTON AGAR SLANTS CONTAINING NO DRUG (MEDIUM O) AND IN CULTURES GROWN ON SAUTON AGAR SLANTS CONTAINING 10 γ OF SULFATHIAZOLE PER ML. OF MEDIUM (MEDIUM ST)

Medium	Age of Cultures	Average Number of Viable Cells Planted for Each Plate	Average Number of Mutants per Each Plate	Mutants per 10^{10} Viable Cells
	days			
O	3	170.2×10^6	36.3	2,140
O	5	125.2×10^6	2.5	200
O	7	96.8×10^6	4.0	414
ST	3	98.6×10^9	0.7	79.0
ST	5	88.0×10^6	0.1	11.4
ST	7	79.6×10^6	1.0	126.0

TABLE 4

ISONIAZID-RESISTANT MUTANTS PER 10^5 VIABLE CELLS OCCURRING IN CULTURES GROWN ON SAUTON AGAR SLANTS CONTAINING NO DRUG (MEDIUM O) AND IN CULTURES GROWN ON SAUTON AGAR SLANTS CONTAINING 10 γ OF SULFATHIAZOLE PER ML. OF MEDIUM (MEDIUM ST)

Medium	Age of Cultures	Average Number of Viable Cells Planted for Each Plate	Average Number of Mutants per Each Plate	Mutants per 10^5 Viable Cells
	days			
O	3	170.2×10^4	92.7	5.45
O	5	125.2×10^4	86.0	6.88
O	7	96.8×10^4	109.0	11.20
ST	3	98.6×10^4	17.0	1.72
ST	5	88.0×10^4	36.4	4.14
ST	7	79.6×10^4	78.4	9.87

bation period, pellicles were formed in both cultures, and the growth seemed to enter into the stationary phase. The growth occurred quite similarily in both cultures.

The numbers of mutants resistant to 10 γ of streptomycin per 10^{10} viable cells and of mutants resistant to 10 γ of isoniazid per 10^5 viable cells were determined in these cultures. The determinations were made in six-, eight-, ten-, and thirteen-day cultures. The results obtained are shown in tables 1 and 2. As shown in table 1, the number of streptomycin-resistant mutants per 10^{10} viable cells in cultures containing 20 γ of sulfathiazole was significantly smaller than that in cultures containing no drug. On the other hand, the number of isoniazid-resistant mutants per 10^5 viable cells in cultures containing the drug was not significantly different from that in cultures containing no drug, as shown in table 2.

Experiment 2: Approximately 10^5 to 10^6 viable cells of the strain were inoculated on Sauton agar slants containing no drug and on Sauton agar slants containing 10 γ of sulfathiazole per ml. of medium and were incubated at 37°C. The growth rates of the strain on these media appeared to be quite similar to each other. After three-, five-, and seven-day

TABLE 5

STREPTOMYCIN-RESISTANT MUTANTS PER 10^{10} VIABLE CELLS AND ISONIAZID-RESISTANT MUTANTS PER 10^{8} VIABLE CELLS OCCURRING IN 6 DAYS' CULTURES GROWN ON SAUTON AGAR PLATES CONTAINING NO DRUG (MEDIUM O) AND IN THOSE GROWN ON SAUTON AGAR PLATES CONTAINING 10 γ OF SULFATHIAZOLE PER ML. OF MEDIUM (MEDIUM ST)

Medium	Age of Cultures	Average Number of Viable Cells Planted for Each Plate	Average Number of Mutants per Each Plate	Mutants per 10^{10}* or 10^{8}† Viable Cells
	days			
(1) Number of Mutants Resistant to 10 γ of Streptomycin per Ml.				
O	6	215.6×10^{6}	21.0	978.0*
ST	6	247.4×10^{6}	0.7	28.4*
(2) Number of Mutants Resistant to 10 γ of Isoniazid per Ml.				
O	6	215.6×10^{4}	51.6	2.39†
ST	6	247.4×10^{4}	72.7	2.94†

TABLE 6

STREPTOMYCIN-RESISTANT MUTANTS PER 10^{10} VIABLE CELLS OCCURRING IN CULTURES GROWN ON SAUTON MEDIUM CONTAINING NO DRUG OBTAINED BY ONE-STEP SELECTION OF 10 γ OF STREPTOMYCIN PER ML. ONLY AND OBTAINED BY ONE-STEP SELECTION OF 10 γ OF STREPTOMYCIN AND 10 γ OF SULFATHIAZOLE PER ML.

Number of Culture	Drugs Used for Selection	Average Number of Viable Cells Planted for Each Plate	Average Number of Mutants per Each Plate	Mutants per 10^{10} Viable Cells
A (*1)	SM	30.8×10^{6}	0.20	65.0
A (*1)	SM + ST	38.8×10^{6}*	0.20	59.2
B (*2)	SM	63.4×10^{6}	3.90	613.0
B (*2)	SM + ST	74.6×10^{6}*	4.00	538.0
C (*3)	SM	166.2×10^{6}	5.60	358.0
C (*3)	SM + ST	180.4×10^{6}*	6.25	347.0

* These numbers of viable cells were those grown on Sauton agar plates containing 10 γ of sulfathiazole per ml. of medium.
(*1): 5-day culture.
(*2): 10-day culture.
(*3): 13-day culture.
SM: streptomycin (10 γ per ml.)
ST: sulfathiazole (10 γ per ml.).

incubation periods, almost the entire growth on the media was collected and the number of streptomycin-resistant mutants and that of isoniazid-resistant mutants per viable cells were determined. The results are shown in tables 3 and 4. The results obtained were similar to the above.

Experiment 5: Approximately thirty to fifty viable cells were inoculated as single cells

TABLE 7

ISONIAZID-RESISTANT MUTANTS PER 10^5 VIABLE CELLS OCCURRING IN CULTURES GROWN ON SAUTON MEDIUM CONTAINING NO DRUG OBTAINED BY ONE-STEP SELECTION OF 10 γ OF ISONIAZID PER ML. ONLY AND OBTAINED BY ONE-STEP SELECTION OF 10 γ OF ISONIAZID AND 10 γ OF SULFATHIAZOLE PER ML.

Number of Culture	Drugs Used for Selection	Average Number of Viable Cells Planted for Each Plate	Average Number of Mutants per Each Plate	Mutants per 10^5 Viable Cells
A (*1)	INH	30.8×10^5	246.0	8.00
A (*1)	INH + ST	$38.8 \times 10^{5*}$	181.0	4.66
A (*1)	INH	30.8×10^4	59.4	19.30
A (*1)	INH + ST	$38.8 \times 10^{4*}$	74.4	19.20
B (*2)	INH	166.2×10^4	142.0	8.55
B (*2)	INH + ST	$180.4 \times 10^{4*}$	157.4	8.73

* These numbers of viable cells were those grown on Sauton agar plates containing 10 γ of sulfathiazole per ml. of medium.
(*1): 5 day-culture.
(*2): 10 day-culture.
INH: Isoniazid (10 γ per ml.)
ST: Sulfathiazole (10 γ per ml.).

on Sauton agar plates containing no drug and on those containing 10 γ of sulfathiazole per ml. of medium and were incubated at 37°C. for six days. Colonies grown on the media were collected, and the appearance rates of mutants were determined as previously mentioned. As shown in table 5, the results obtained were quite similar to the above.

In this experiment, the incubation period to discover visible colonies and the size of colonies on the media were quite similar to each other in the presence as well as in the absence of sulfathiazole. Therefore, the growth phase of cultures appeared to be similar to each other.

Experiment 4: With the purpose of examining the possibility that the mutation to streptomycin resistance or to isoniazid resistance can be related to the mutation to sulfathiazole susceptibility (reverse mutation) or not, the following experiments were performed. The appearance rate of mutants obtained by selection of streptomycin alone or isoniazid alone was compared with that obtained by selection of both streptomycin and sulfathiazole or both isoniazid and sulfathiazole. As shown in tables 6 and 7, the numbers of mutants obtained by both methods of selection were not different from each other. These results indicate that the growth of streptomycin-resistant mutants and isoniazid-resistant mutants are not inhibited and not delayed by the presence of sulfathiazole and that streptomycin-resistant mutants or isoniazid-resistant mutants isolated are always mutants simultaneously resistant to both streptomycin and sulfathiazole or mutants resistant to both isoniazid and sulfathiazole.

* * *

The results indicate that the appearance rate of streptomycin-resistant mutants in cultures containing 10 to 20 γ of sulfathiazole per ml. of medium is significantly smaller than that in cultures containing no drug. Therefore, it would seem that the mutation rate to streptomycin resistance in the strain is much smaller in the presence of sulfathiazole than in the absence of the drug, if the growth rate of streptomycin-resistant mu-

tants, i.e., mutants simultaneously resistant to both streptomycin and sulfathiazole, were similar to that of nonmutants, i.e., mutants resistant to sulfathiazole alone. It has been shown in *Experiment 4* that the growth rates are similar to each other. Therefore, it would be concluded that the mutation rate to streptomycin resistance in the sulfathiazole-resistant strain is smaller in the presence of sulfathiazole (10 to 20 γ per ml.) than in the absence of the drug. In contrast, the mutation rate to isoniazid resistance is not affected by the presence of sulfathiazole.

The concentration of sulfathiazole added to cultures in the study never appeared to be inhibitory for the growth of all individual cells of the strain (*Experiment 4*). The fact that the presence of sulfathiazole which was not selective for any individual cells decreased the mutation rate to streptomycin in the sulfathiazole-resistant strain appears to be remarkable. The mechanism of the phenomenon is not known, but it may be possible that this is related to the mechanism of mutations themselves.

Klein and Kimmelman[7] reported on the effect of sulfadiazine in preventing the emergence of streptomycin resistance in *Staphylococcus aureus* and interpreted their results on the basis of the selection and inhibition of resistant mutants. Tsukamura, Suzuki, and Kimino[8] suggested that the effect of sulfathiazole in preventing the emergence of streptomycin resistance in *Mycobacterium avium* was not interpreted on the basis of the selection of resistant mutants only. It appeared to be required for the interpretation of the effect to postulate another mechanism as an effect of the drug in changing the mutation rate to streptomycin resistance. In another paper, which is published concurrently,[2] the writer reported on the decrease of mutation rate to streptomycin resistance in a sulfathiazole-resistant strain of *Mycobacterium avium*. The results shown in the present paper seem to indicate that the decrease of mutation rate to streptomycin resistance in sulfathiazole-resistant mutants produced by the presence of sulfathiazole occurs on a nonselective basis. Thus, the phenomena previously observed[1, 5] and the mechanism of action of sulfathiazole in preventing the emergence of streptomycin resistance appear to be better interpreted by the results shown in these reports.

* * *

The effect of sulfathiazole on mutations to streptomycin resistance and to isoniazid resistance has been studied in a sulfathiazole-resistant strain of *Mycobacterium avium*. The appearance rate of streptomycin-resistant mutants and of isoniazid-resistant mutants was determined in cultures containing no drug and in cultures containing sulfathiazole (10 to 20 γ per ml.). The appearance rate of streptomycin-resistant mutants was significantly smaller in the presence of sulfathiazole than in the absence of sulfathiazole, whereas the appearance rate of isoniazid-resistant mutants was not significantly affected by the presence of sulfathiazole. Growth rates of nonmutants, streptomycin-resistant mutants, and isoniazid-resistant mutants appeared to be quite similar to each other in the presence of sulfathiazole as well as in the absence of sulfathiazole. The concentrations of sulfathiazole added to media were never inhibitory for any individual cells of population of the strain and, therefore, never appeared to be selective.

In summary, it has been concluded that the mutation rate to streptomycin resistance in the sulfathiazole-resistant strain of *Mycobacterium avium* is significantly smaller in the presence of sulfathiazole than in the absence of sulfathiazole. The mutation to streptomycin resistance in the strain is significantly decreased by the presence of sulfathiazole,

[7] Klein, M., and Kimmelman, L. J.: J. Bact., 1947, *54*, 363.
[8] Tsukamura, M., *et al.*: J. Antibiot., *Series B*, 1955, *8*, 409.

whereas the mutation rate to isoniazid resistance in the strain is not affected by the presence of sulfathiazole. Thus, the existence of a specific effect of sulfathiazole in reducing the emergence of streptomycin resistance by a nonselective mechanism has been indicated.

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TUBERCULOUS INFECTION DURING ACADEMIC STUDIES

Conversion of the tuberculin skin test reaction from negative to positive is generally accepted as an evidence of infection with tubercle bacilli. Smith[1] states: "A recent conversion to positive tuberculin reaction means that there is a known or unknown active case among the converter's associates." There are experts who advocate chemotherapy with antituberculous drugs in cases of recent "conversion" without other clinical or laboratory evidence of disease.[2, 3] This recommendation indicates that there is no doubt in the minds of the proponents of treatment that conversion to a positive skin reaction to tuberculin means infection with *M. tuberculosis*.

The mortality rate of tuberculosis has been decreasing since the initiation of antimicrobial therapy, which started with the general availability of streptomycin in 1947.[4] However, the morbidity rate does not show the same decline.[5] In light of these facts it would appear interesting to learn the rate of tuberculous infection in an academic community and the tuberculosis exposure and infection rate among university students. It cannot be denied that the number of tuberculin reactors is declining[6] among students, but the tuberculosis problem is still very much present in universities.[7]

Tuberculin skin reactions of 4,634 University of Chicago students registered in 1955 were analyzed. The survey included undergraduate and graduate students, some of them with six or seven previously negative tuberculin reactions and some students who had never before had skin tests. The tuberculin reaction was tested routinely in every year on all students except those who converted to positive reactions. Excluded from the study were known cases of tuberculosis, students with a previous history of tuberculosis, with a history of previously positive skin tests, and with a history of BCG immunization. Many of the foreign-born students reported previously positive skin tests. These subjects were not included in the present study. Omitting from the study this group of most probably positive cases decreased the proportion of foreign-born reactors but increased the reliability of the data. As will be shown later, in spite of this exclusion, the number of reactors was significantly higher among the foreign born than among North Americans. Those born in the continental United States or in Canada were classified as North Americans. The term Latin-Americans includes persons with place of birth stated to be south of the United States. Every student had a chest microfilm concurrently with the skin test, and all the students with abnormal findings in the microfilm had 14- by 17-inch stereoscopic chest films.

The tuberculin reaction and the conversion rate of the general student population and of the medical students were analyzed according to the following categories: sex; origin (birthplace); race (no attempt was made to analyze the students according to national extractions); the age of subjects at the time of conversion; the academic year of conversion (length of time spent in academic community prior to the conversion); season of conversion; roentgenographic findings, and differential leukocyte counts and erythrocyte sedimentation rates at the time of conversion.

The skin test was performed with 0.1 ml. of tuberculin PPD (purified protein derivative) intradermally in the forearm and was read after forty-eight hours. It was considered positive if the induration was at least 5 mm. and the erythema at least 10 mm. in diameter.

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[1] Smith, D. T.: Dis. of Chest, 1954, *26*, 614.
[2] McDermott, W., and Youmans, G.: Am. Rev. Tuberc., 1953, *63*, 819.
[3] Waring, J. J.: Dis. of Chest, 1954, *25*, 361.
[4] Peabody, J. W., Sr.: Minnesota Med., 1956, *5*, 312.
[5] Drolet, G. J., and Lowell, A. M.: Am. Rev. Tuberc., 1955, *72*, 419.
[6] Krug, E. S., and Gleen, H. R.: Journal-Lancet, 1955, *75*, 132.

TABLE 1

TUBERCULIN REACTIONS ACCORDING TO PLACE OF BIRTH (ORIGIN) AND RACE

Category of Students	Total Number	Negative at Entrance		Positive at Entrance		Converted from Negative		Positive at End	
		Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Total number of students	4,634	3,712	80.1	922	19.9	198	5.3	1,120	24.1
American white	3,875	3,346	86.3	529	13.7	158*	4.7	687	17.5
American Negro	154	98	63.6	56	36.4	11	11.2	67	43.5
American Oriental	17	11	64.7	6	35.3	0	0	6†	35.3
American total	4,046	3,455	85.4	591	14.6	169‡	4.8	760	19.0
Foreign-born white	382	186	48.6	196	51.4	20*	10.7	216	56.5
Foreign Oriental	189	64	33.8	125	66.2	9	14.0	134†	70.8
Foreign African	9	3	33.3	6	66.7	0	0	6	66.7
Latin-American foreign									
Non-white foreign	8	4	50.0	4	50.0	0	0	4	50.0
Foreign total	588	257	43.8	331	56.2	29‡	11.2	360	61.2

* $\chi^2 = 7.74$; $p < 0.01$.
† $\chi^2 = 11.07$; $p < 0.01$.
‡ $\chi^2 = 19.30$; $p < 0.01$.
Differences are statistically significant.

The skin test solution was prepared in such a way that 0.1 ml. contained 0.0001 mg. of tuberculin (PPD).

Analyzing the results, it was found (table 1) that 80.1 per cent of the population tested was negative at the entrance examination. The development of cutaneous sensitivity to tuberculin occurred in 198, or 5.3 per cent, of the students. At the end of this study the number of positive reactors was 1,120 (24.1 per cent) compared with 922 (19.9 per cent) at the start of their academic study.

A statistically significant difference was found in the conversion rate among the North American-born and foreign-born students. The conversion was only 4.8 per cent among the North American students and 11.2 per cent among the foreign-born. This occurred in spite of the fact that the percentage of positive reactors at the time of entrance examination was 14.6 per cent among North American students and 56.2 per cent among foreign-born students.

It was unexpected and puzzling to find more than twice as many *converters* among the foreign-born as among the North American-born. Climate, nutrition, and the possibility of exposure to infection in the academic environment should certainly be the same for both groups. The infection took place in the United States where the frequency and possibility for exposure to tuberculosis are lower than in the native lands of the foreign-born. There is no satisfactory explanation for this unexpected finding. The same statistically significant difference can be detected in comparing the conversion rates of North American-born white and foreign-born white students (table 1, 4.7 per cent and 10.7 per cent, respectively).

The tuberculin reaction was positive in 35.3 per cent of the North Americans of Asiatic

TABLE 2

TUBERCULIN REACTIONS ACCORDING TO SEX, RACE, AND PLACE OF BIRTH

Category of Students	Total Number	Negative at Entrance		Positive at Entrance		Converted from Negative		Positive at End	
		Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Total number of students	4,634	3,712	80.1	922	19.9	198	5.3	1,120	24.1
Male	3,465	2,771	80.0	694	20.0	170*	6.1	864	24.9
Female	1,169	941	80.5	228	19.5	28*	2.9	256	21.8
American white male	2,927	2,519	86.1	408	13.9	139†	5.5	547	18.6
American white female	948	827	87.3	121	12.7	19	2.2	140	14.7
American Oriental male	11	6	54.5	5	45.5	0	0	5	45.5
American Oriental female	6	5	83.4	1	16.6	0	0	1	16.6
American Negro male	90	58	64.5	32	35.5	8†	13.7	40	44.4
American Negro female	64	40	62.5	24	37.5	3	7.5	27	42.1
American total males	3,028	2,583	85.4	445	14.6	147	5.6	592	19.5
American total females	1,018	872	85.7	146	14.3	22	2.5	168	16.5
Total American	4,046	3,455	85.4	591	14.6	169	4.8	760	19.0
Foreign-born white male	283	137	48.1	146	51.9	16	11.6	162	57.2
Foreign-born white female	99	49	49.4	50	50.6	4	8.1	54	54.5
Foreign Oriental male	139	45	32.4	94	67.6	7	15.5	101	72.6
Foreign Oriental female	50	19	38.0	31	62.0	2	10.5	33	66.0
Foreign African male	8	2	25.0	6	75.0	0	0	6	75.0
Foreign African female	1	1	100.0	0	0	0	0	0	0
Latin-American non-white male	7	4	57.1	3	42.9	0	0	3	42.9
Latin-American non-white female	1	0	0	1	100.0	0	0	1	100.0
Foreign-born total males	437	188	43.1	249	56.9	23	12.2	272	62.2
Foreign-born total females	151	69	45.6	82	54.4	6	8.6	88	58.2
Total foreign born	588	257	43.8	331	56.2	29	11.2	360	61.2

* $\chi^2 = 12.02$; $p < 0.01$.

† $\chi^2 = 8.80$; $p < 0.01$.

Differences are statistically significant.

descent at the end of the study and in 70.8 per cent of the Asiatic-born group, which difference is again highly significant statistically (table 1). Similar findings have been reported by Lees[7] in active tuberculous cases among young adults.

Reactions to tuberculin according to sex are presented in table 2. Six and one-tenth per cent of the male population were tuberculin "converters" as compared with 2.9 per cent of the female students. This striking difference by sex for the 4,634 students in the entire group occurs for all subgroups regardless of places of birth.

The conversion rate for the North American white male and the North American Negro male population is 5.5 per cent and 13.7 per cent, respectively, which is again a significant difference (table 2).

[7] LEES, H. D.: Journal-Lancet, 1954, *74*, 120.

TABLE 3

DISTRIBUTION OF SUBJECTS ACCORDING TO ORIGIN, SEX, AND RACE

Category of Student	Total Number	Male		Female	
		Number	Per Cent	Number	Per Cent
Total number of students	4,634	3,465	74.7	1,169	25.3
American white	3,875	2,927	75.5	948	24.5
American Oriental	17	11	64.8	6	35.2
American Negro	154	90	58.5	64	41.5
American total	4,046	3,028	74.9	1,018	25.1
Foreign-born white	382	283	74.1	99	25.1
Foreign Oriental	189	139	73.6	50	26.4
Foreign African	9	8	88.8	1	11.2
Latin-American non-white	8	7	87.5	1	12.5
Foreign-born total	588	437	74.4	151	25.6

The numerical and percentagewise distribution of subjects according to sex, race, and origin is presented in table 3. Of the population examined, about 25 per cent were females. Eighty-seven and three-tenths per cent of the students were North American and 12.7 per cent were foreign born.

The tuberculin reaction was positive in 19.0 per cent of the North Americans and in 61.2 per cent of the foreign-born students at the end of this study. Thus, the prevalence of reactions for North Americans was less than one-third that among the foreign born. Only seven years prior to this study, Loosli and co-workers[8] found at the same university that this rate was less than one-half (24.8 per cent to 54.6 per cent). These data prove that, while cutaneous sensitivity to tuberculin has decreased among North Americans, it has increased slowly among foreign-born students during the last seven years. In Loosli's study of 13,127 students, almost 10 per cent came from foreign countries while in the present study almost 13 per cent were foreign born.

It is well known from the studies of Myers[9, 10] and others[11, 12] that nurses, medical students, and physicians are more exposed to and infected with tuberculosis than other groups.

In comparing the medical student group with the entire group of students, it should be noted that the entire group is composed of 75 per cent males, and the medical student group consists of more than 90 per cent males. It was found in this study that, while medical students (table 4) had a lower percentage (14.2 per cent) of positive tuberculin reactions than the total student body (19.9 per cent) at the beginning of their studies, the percentage of *conversion* was 11.3 per cent among medical students compared with 5.3 per cent among the whole student population and 4.8 per cent among the nonmedical stu-

[8] LOOSLI, L. G., *et al.*: Am. J. Hyg., 1951, *53*, 33.

[9] MYERS, J. A.: Tuberculosis Among Children and Adults, ed. 3, Charles C Thomas, Springfield, Illinois, 1951, pp. 525, 537, and 545.

[10] MYERS, J. A., *et al.*: J.A.M.A., 1955, *158*, 1.

[11] VERNAY, R. E.: Brit. M. J., 1955, No. 4945, 929.

[12] MEADE, G. M.: Am. Rev. Tubere., 1948, *58*, 675.

TABLE 4

TUBERCULIN REACTION OF MEDICAL STUDENTS ACCORDING TO SEX AND ORIGIN

Category of Student	Total Number	Negative at Entrance		Positive at Entrance		Converted from Negative		Positive at End	
		Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Total number of students	297	255	85.8	42	14.2	29	11.3	71	23.9
Males	276	241	87.4	35	12.6	25	10.3	60	21.7
Females	21	14	66.7	7	33.3	4	28.5	11	52.3
American male	241	218	90.5	23	9.5	21	9.6	44	18.2
American female	16	14	87.5	2	12.5	4	28.5	6	37.5
American total	257	232	90.2	25	9.8	25	10.7	50	19.4
Foreign-born male	35	23	65.8	12	34.2	4	17.3	16	45.7
Foreign-born female	5	0	0	5	100.0	0	0	5	100.0
Foreign-born total	40	23	57.5	17	42.5	4	17.3	21	52.5

dent population. Here again, the difference is statistically highly significant ($\chi^2 = 18.3$, $p < 0.01$). Thus, in spite of the fact that the medical student body started with a lower percentage of reactors, by the end of the university period this group had developed about the same percentage of reactors (23.9 per cent) as the total student population (24.1 per cent). The foreign-born were represented in about the same proportion (13.4 per cent) as in the general student population. The high incidence of conversion rate of the female medical students is noteworthy, compared with the total female student population. The same is true for the high total percentage of positive reactors among female medical students at the end of the study.

Loosli and co-workers[8] proved that the rate of tuberculin sensitivity increased with age. In the present study the number of male converters was the highest (57.6 per cent) in the age bracket of twenty-one to thirty years, while the conversion of females exhibited a more even distribution in different age groups (table 5). The percentage distribution of the new students by age groups was about the same[13] in 1955 as the conversion rate in the studied population, e.g., 27 per cent of the new students were less than twenty years old, and the percentage of conversion was 26.7 in the same age group of the entire student population.

Investigating the rate of conversion according to duration of time spent at the university, it was found that only a very negligible number (1.5 per cent) of students became cutaneous reactors to tuberculin during the first year of their academic studies (table 6). Almost one half (45.4 per cent) of conversions occurred during the second academic year. The "conversion" rate was still high after the students had spent five or more years on the campus; 17.1 per cent of all "conversions" took place after five years of academic study.

The 198 conversions occurred during seven years, 77 per cent of which took place during the last two years of survey (1954 and 1955) and 90 per cent occurred between 1952 and 1955. This study and the conversion rate of 5.3 per cent include all students who were registered in 1955 irrespective of the date of their first enrollments. In table 6

[13] HERBOLSHEIMER, H.: Personal communication.

TABLE 5

AGE AT THE TIME OF CONVERSION

Years of Age	Total Number	<20		21–30		31–40		More than 40	
		Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Total number of students...	198	53	26.7	109	55.0	29	14.6	7	3.5
Male.....	170	45	26.4	98	57.6	23	13.5	4	2.3
Female.....	28	8	28.5	11	39.2	6	21.4	3	10.7

TABLE 6

ACADEMIC YEAR OF CONVERSION

Year of Conversion	Total Number	First		Second		Third		Fourth		Fifth or More	
		Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Total number of students..	198	3	1.5	90	45.4	53	26.7	18	9.0	34	17.1
Male.....	170	3	1.7	77	45.2	46	27.0	15	8.8	29	17.0
Female.....	28	0	0	13	46.4	7	25.0	3	10.7	5	17.8

TABLE 7

SEASON OF CONVERSION

Season	Total Number	Fall		Winter		Spring		Summer	
		Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Total number of students..	198	100	50.5	57	28.7	23	11.6	18	9.0
Male.....	170	88	51.7	48	28.2	19	11.1	15	8.8
Female.....	28	12	42.8	9	32.1	4	14.2	3	10.7

it may be noted that 82.6 per cent of the conversions took place in the first four years and 72.1 per cent during the second and third years of the academic study.

Most of the "conversions" (50.5 per cent) were detected during the autumn season and the fewest took place during the summer (table 7). Since the enrollment of new students is highest in the fall quarter and skin testing usually was repeated exactly one year after the first testing, this finding cannot prove or disprove the statements of De Rudder and others [14] that tuberculin sensitivity decreases during autumn and increases during the spring.

The roentgenographic examinations (microfilms and large [14- by 17-inch] films) revealed only one case of active tuberculosis among the 198 tuberculin converters. Chest microfilms were taken of all 198 "converters" at the time of conversion and every three months thereafter for one year. Negative chest microfilms were reported for 190 converters (95.9 per cent), insignificant findings (artefacts, adhesions, calcifications, et cetera) were reported in 7 (3.5 per cent). Large-size roentgenograms were taken of 41 converters, only

[14] DE RUDDER, B.: Grundriss einer Meteorobiologie des Menschen. Dritte Auflage Springer Verlag, Berlin-Göttingen-Heidelberg, 1952, pp. 226–227.

one of whom turned out to have active tuberculosis; it was the same patient in whom the lesion had already been detected by microfilm.

Complete blood counts were done in 72.2 per cent of the converters. Seventy-five converters (52.4 per cent) had lymphocytosis. Relative lymphocytosis (40 to 50 per cent) was encountered in 37 per cent of the converters and absolute lymphocytosis in 15.4 per cent of the patients. Erythrocyte sedimentation rate (Wintrobe method) was determined in 108 cases of conversion. In 7 cases (6.4 per cent) it was more than 20 mm. Kneller,[15] in his interesting and critical review on blood pictures in tuberculosis, quotes among others Cerami whose opinion is: "A general improvement in pulmonary tuberculosis was associated with a lymphocytosis, mild degree of eosinophilia, decrease in neutrophiles, reduced E.S.R. and shift to the right." One can speculate whether lymphocytosis in case of tuberculin conversion has the same favorable significance as in pulmonary tuberculosis.

In summary: The conversion rate among 4,634 university students to a positive tuberculin skin reaction was 5.3 per cent during their academic studies. The conversion rate of foreign-born students was statistically significantly higher than that of North American-born students. The same is true for the white foreign-born converters compared with white North American converters. More than twice as many male as female students converted to positive. The conversion rate among North American white males was also significantly higher than among females. The percentage of conversion was significantly lower among North American white males than among North American Negro male students. American-born Orientals have had a significantly lower incidence of positive tuberculin test than the Asian-born students. The tuberculin positivity decreased among North Americans and increased among foreign-born students during the last seven years.

Medical students have had a significantly higher conversion rate than the nonmedical student population.

Most conversions occurred during the second year of academic study, and none of the converters developed clinical disease after the conversion. More than half of the converters examined had lymphocytosis.

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February 21, 1957

[15] Kneller, F.: Tubercle, 1954, *35*, 31.

THE EVALUATION OF THE EGG EMBRYO AS A LABORATORY PROCEDURE FOR THE RAPID DETECTION OF THE TUBERCLE BACILLUS

There is a great need for more rapid detection of *Mycobacterium tuberculosis* in specimens obtained from patients than is afforded by regular culture methods.

In recent years numerous articles have appeared on various phases of demonstrating tubercle bacilli in the embryonated egg.[1-12] The present writers wished to test the fertilized hen's egg as a procedure in the diagnosis of tuberculosis and to simplify the technique so that it could be used in a routine clinical laboratory.

Preliminary experiments with pure cultures isolated from clinical material and a pure pathogenic test strain (H37Rv) gave consistently positive results. These indicated that the site of injection and the egg materials examined were satisfactory for demonstration of acid-fast bacilli.

After trying the yolk emulsion technique described by Brueck and Buddingh[13, 14] for examination of the eggs, artefacts resembling acid-fast bacilli were often found in the microscopic slide preparations. In figure 1 of yolk suspension artefacts resembling acid-fast bacilli may be noted, as compared with figure 2 of allantoic material in which distinct bacilli are shown. Examinations were made not only of the yolk emulsion, but also of the allantoic materials. These consisted of sediment, surface film, and the histologic preparations of the allantoic membrane.[15] Subsequently, additional portions of the chick embryo were examined. Slides made of surface film and sediment of the allantoic membrane were easier to interpret than those of the yolk emulsion. The preparation of the histologic membrane slides was time consuming, and the microscopic findings when compared with those of the other egg materials were not conclusive. For these reasons such preparations are not recommended for routine use.

The injection was made through a small drill hole into the chorioallantoic cavity directly below the air sac. Inoculum was 0.2 to 0.5 ml. for each egg. Having established a satisfactory technique with reliable results, using these pure cultures, six- to eight-day-old embryos were inoculated with concentrated human materials. The concentration procedure was that described in Tuberculosis Laboratory Methods, Fourteenth Conference

[1] Hsiung, G. D., and Haley, L. D.: Am. Rev. Tuberc., 1954, *70*, 912.
[2] Goodpasture, E. W., and Anderson, K.: Am. J. Path., 1937, *13*, 149.
[3] Emmart, E. W., and Smith, M. I.: Pub. Health Rep., 1941, *56*, 1277.
[4] Moore, M.: Am. J. Path., 1942, *18*, 827.
[5] Emmart, E. W., and Smith, M. I.: Am. Rev. Tuberc., 1943, *47*, 426.
[6] Fite, G. L., and Olson, B. J.: Pub. Health Rep., 1944, *59*, 1423.
[7] Eggerth, A. H., *et al.*: Am. Rev. Tuberc., 1948, *57*, 632.
[8] Bunn, P. A., *et al.*: J. Lab. & Clin. Med., 1955, *45*, 658.
[9] McNelly, E., and Riddell, W. A.: Am. J. Pub. Health, 1949, *39*, 1463.
[10] Lee, H. F.: Ann. New York Acad. Sc., 1949, *52*, 692.
[11] Canat, E., and Opie, E.: Am. J. Path., 1943, *19*, 385.
[12] Francis, J.: Am. Rev. Tuberc., 1956, *73*, 276.
[13] Brueck, J. W., and Buddingh, G. J.: Proc. Soc. Exper. Biol. & Med., 1952, *80*, 589.
[14] Buddingh, G. J., and Brueck, J. W.: J. Louisiana M. Soc., 1953, *105*, 304.
[15] The allantoic sediment and surface film were formed by suspending overnight the allantoic membrane in a fixing solution of formalin. Smears were made of the top film that formed on the liquid. Sediment smears were made from material that remained in the bottom of the tube after the allantoic membrane was removed for histologic processing.

FIG. 1. Photomicrograph of yolk suspension showing artefacts resembling acid-fast bacilli.

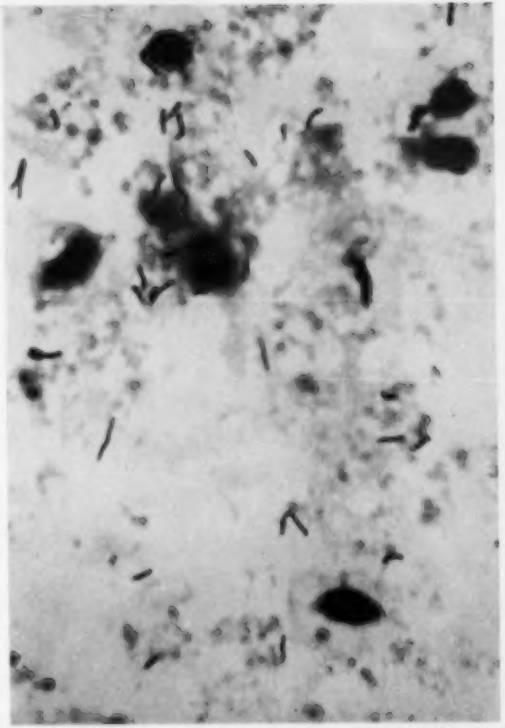

FIG. 2. Photomicrograph of allantoic material showing distinct presence of acid-fats bacilli.

TABLE 1*A*

COMPARISON BY SPECIMENS OF ROUTINE LABORATORY EXAMINATIONS AND EGG EMBRYOS INOCULATED WITH CONCENTRATED HUMAN MATERIAL*
(116 Specimens)

	Routine Laboratory Findings	Yolk		Allantoic Top		Allantoic Sediment	
		Positive	Negative	Positive	Negative	Positive	Negative
Positive culture,† positive microscopy	35	17	18	23	12	23	12
Positive culture, negative microscopy	11	7	4	3	8	9	2
Negative culture, positive microscopy	13	6	7	7	6	10	3
Negative culture, negative microscopy	57	13	44	13	44	16	41

* Positive or negative for *M. tuberculosis* is the final result of the combination of the six eggs in the series.

† Routine cultures made on Löwenstein-Jensen egg medium.

TABLE 1*B*

COMPARISON BY PATIENTS OF ROUTINE LABORATORY EXAMINATIONS AND EGG EMBRYOS INOCULATED WITH CONCENTRATED HUMAN MATERIAL*
(49 Patients)

	Routine Laboratory Findings	Yolk		Allantoic Top		Allantoic Sediment	
		Positive	Negative	Positive	Negative	Positive	Negative
Positive culture,† positive microscopy	17	12	5	13	4	12	5
Positive culture, negative microscopy	4	1	3	1	3	2	2
Negative culture, positive microscopy	4	2	2	3	1	2	2
Negative culture, negative microscopy	24	6	18	6	18	8	16

* Positive or negative for *M. tuberculosis* is the final result of the combination of the six eggs in the series.

† Routine cultures made on Löwenstein-Jensen egg medium.

TABLE 2

COMPARISON OF ACID-FAST BACILLI IN ROUTINE LABORATORY EXAMINATIONS AND THE EGG EMBRYO

(By Specimens)	Routine Laboratory Findings	Positive Egg	Negative Egg
Positive* culture, positive microscopy	35	25	10
Positive culture, negative microscopy	11	9	2
Negative culture, positive microscopy	13	10	3
Negative culture, negative microscopy	57	18	39
Total specimens	116		

(By Patients)		
Positive routine laboratory findings, embryo positive	25	Total 30: positive findings
Positive routine laboratory findings, embryo negative	5	
Negative routine laboratory findings, embryo positive	4	Total 19: negative findings
Negative routine laboratory findings, embryo negative	15	
Total patients	49	

* Positive for *M. tuberculosis*.

TABLE 3

AREAS SHOWING ACID-FAST ORGANISMS IN 62 POSITIVE EMBRYOS

Areas of Egg	Positive Results
Yolk material only	1
Allantoic* top only	3
Allantoic† sediment only	5
Allantoic top and sediment (not yolk)	12
Allantoic sediment and yolk (not allantoic top)	15
Allantoic top and yolk (not allantoic sediment)	2
Yolk, allantoic top and allantoic sediment	24

* Film that forms on top of formalin in which allantoic membrane has been suspended for twenty-four hours.

† Sediment that forms on the bottom of formalin tube in which allantoic membrane has been suspended for twenty-four hours.

on The Chemotherapy of Tuberculosis.[16] One hundred and sixteen concentrated specimens representing 49 different patients were inoculated into each of a series of six embryos.

In table 1*A*, the data accumulated from the individual specimens are summarized. In table 1*B* the same results are summarized in relation to patients.

The number of positive eggs (13 to 16 instances) in table 1*A* in which the results were negative by the routine examinations is noteworthy. In table 2 a comparison of acid-fast bacilli in the routine laboratory examination with those in the egg embryo may be seen. There were 18 positive eggs in which routine laboratory studies were negative. Areas of the egg embryo that were positive for acid-fast organisms are shown in table 3. Twelve times the organisms were found in the allantoic material and not in the yolk. In 24 instances organisms were found in all of the allantoic materials and in the yolk. Only one time was the yolk the only area that showed infection.

* * *

In this study, the longest period of incubation before final results were determined was ten to twelve days. Most routine culture techniques require a longer time to produce positive growth. From the results obtained, it cannot be said that the fertilized egg is a more sensitive medium. However, of 57 negative specimens by routine microscopic and culture examinations, 18 showed acid-fast bacilli in the egg. These 18 specimens represent 4 patients with supposedly noninfectious sputum as determined by all routine laboratory examinations. There were 15 of 59 specimens that gave negative egg results when routine smears or cultures were positive for *M. tuberculosis*. For the remaining specimens the egg embryo findings paralleled the routine findings, but the results in the eggs were available at an appreciably earlier date.

In summary: The fertilized hen's egg can be utilized in a clinical laboratory for the rapid diagnosis of tuberculosis. Ten to twelve days of incubation after injection proved sufficient time for recovery of the organism in the egg embryo. Cultures negative for tubercle bacilli by the routine laboratory method may be positive in the egg embryo. Slides were easier to interpret when made from the surface film and sediment in which the allantoic membrane had been suspended rather than from the yolk emulsion. Micro-

[16] Tuberculosis Laboratory Methods, Tr. Fourteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1955, p. 514.

scopic examination of the allantoic material revealed acid-fast bacilli as often as did the yolk emulsion slides. The allantoic tissue section slides were of no greater value than the other egg materials examined; therefore, the histologic work involved would not justify this preparation. The egg embryo is a good culture medium and may prove to be an adjunct for the rapid diagnosis of tuberculosis.

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January 17, 1957

[17] The writers wish to express their appreciation to Dr. Robert V. Thomson, Chief, Clinical Laboratory Service, for his suggestions, and to Miss Lorraine Kelly, technician, for her assistance in the histologic preparation of the slides.

THE VITAL CAPACITY IN BRONCHOSPIROMETRY[1]

In the analysis of the bronchospirometric records, the distributions of functions and volumes are expressed as percentage of the total. In the study of the oxygen uptake and minute ventilation of the individual lung, this method has been necessary, since the absolute values have little significance. In the study of the distribution of the vital capacity in bronchospirometry, the percentage of the total has been the method of expression. The vital capacities are lung volumes that in their absolute terms do have significance, since the sum of right plus left vital capacity found in bronchospirometry is within plus-minus 10 per cent of the vital capacity as found spirometrically (PINNER, M., *et al.*: J. Thoracic Surg., 1942, *11*, 241). In several studies made on normals, the distribution of the right to the left lung has been shown to be in a 55 to 45 ratio.

It appears that reporting only the percentage of the total for the vital capacity of each lung could lead to misconception about the remaining function of the lung if at the same time the percentage of the predicted value for the total spirometry is not included. Therefore, it is proposed that, in addition to recording the percentage of the total for the vital capacities in bronchospirometry, the percentage of the predicted vital capacity for each lung also be recorded.

The formula for the percentage of predicted vital capacity for the right lung is as follows:

$$\text{Per cent of predicted vital capacity (right lung)} = \frac{\text{observed vital capacity (right lung)} \times 100}{0.55 \times \text{(predicted vital capacity, both lungs)}}$$

In the normal the following information would be included: (*1*) Vital capacity was 100 per cent of predicted, with a distribution of 45 per cent of the total for the left lung and 55 per cent of the total for the right lung; (*2*) vital capacity, right lung, 100 per cent of predicted for the right lung; (*3*) vital capacity, left lung, 100 per cent of predicted for the left lung.

In pathologic cases the following examples will illustrate the value of such additional information.

(*1*) *Case 100:* The roentgenogram of this patient shows fibrocaseous tuberculosis involving the left lung. Below the left clavicle there is a cavity, measuring 1.5 cm. There is another cavity in the left first anterior interspace. The left leaf of the diaphragm is elevated, suggesting a pleural reaction. The heart and mediastinum are displaced to the left. A scoliosis to the right is noted in the dorsal region.

On spirometry the vital capacity was found to be 4,260 cc., or 96 per cent of predicted. On bronchospirometry the right lung was found to have 79 per cent of the total vital capacity and the left lung, 21 per cent of the total vital capacity.

When these figures are calculated with respect to the percentage of predicted for each lung, it is found that the value for the right lung is 124 per cent of that predicted for the right lung, and the value for the left lung is 39 per cent of that predicted for the left lung. These figures now reflect the stretching of the right lung to compensate for the shift of the mediastinum to the left.

[1] Since submission of this method of calculation, our attention has been called to the following papers: LIMBERG, M.: Acta tuberc. Scandinav., 1954, *29*, 237; and BIRATH, G., *et al.*: Am. Rev. Tuberc., 1957, *75*, 699.

TABLE 1

VITAL CAPACITY IN A SERIES OF BRONCHOSPIROMETRIC STUDIES

Number	Spirometry	Bronchospirometry			
		Right Lung, Vital Capacity		Left Lung, Vital Capacity	
	Per Cent of Predicted Total Vital Capacity	Per Cent of Total	Per Cent of Predicted	Per Cent of Total	Per Cent of Predicted
72	66	63	67	37	47
88	87	76	111	24	43
94	61	62	72	38	53
100	96	79	124	21	39
139	81	34	52	66	121
144	106	55	96	45	94
166	55	64	55	36	39
185	42	92	69	8	7
269	76	30	39	70	113
325	73	76	91	24	35
353	62	39	43	61	83
429	65	31	38	69	105
456	125	55	126	45	126
476	110	38	76	62	149

(*2*) *Case 185:* The roentgenogram of this patient shows an extensive left upper thoracoplasty. There are thickened pleural scars overlying the base of the left diaphragm. The right upper lobe region reveals strands of increasing densities, probably representing at least two fairly large-sized cavity formations.

On spirometry the vital capacity was found to be 1,800 cc., or 42 per cent of predicted. On bronchospirometry the right lung was found to have 92 per cent of the total vital capacity and the left lung 8 per cent of the total vital capacity.

When these figures are calculated as to the per cent of predicted for each lung, it is found that the value for the right lung is 69 per cent of that predicted for the right lung, and the value for the left lung is 7 per cent of that predicted for the left lung. With these figures it becomes obvious that the right lung has lost about 30 per cent of its vital capacity and the left lung has lost more than 90 per cent of its vital capacity.

In table 1 the figures are given of the vital capacity in a series of bronchospirometric studies when calculated according to the method suggested above.

In summary: It is suggested that the vital capacity of each lung as found by bronchospirometry be calculated in percentage of the vital capacity predicted for the respective lung. This would show more clearly the presence of an abnormal vital capacity of one lung.

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Books

OBSERVATIONS ON KREBIOZEN IN THE MANAGEMENT OF CANCER. By A. C. Ivy, J. F. Pick, and W. F. P. Phillips. Pp. 88, Henry Regnery Co., Chicago, 1956. Price, $2.50.

This, the third monograph to emanate from the Krebiozen Research Foundation in Chicago, is in essence an amplified reiteration of theories and poorly documented findings reported in 1951 and 1954.

In a brief introduction, the authors review the highly controversial literature on the body's natural resistance to malignant disease. It is concluded by them that the reticuloendothelial system "because of the numerous defensive and repair mechanisms it manifests, *might* be suspected to play a role in determining the resistance of the body to the development, invasion and metastatis of neoplastic growth."[1] On the basis of this unproved hypothesis, Dr. Steven Durovic prepared his "antiblastic substance" which he called "Krebiozen." In the current report we are told that the chemical structure of Krebiozen still remains to be determined. It is noteworthy, but not stressed in this report, that the method of extraction of the compound from horse serum and its purification are still in the hands of Dr. Durovic as "a business secret." Apparently no efforts have been made since 1948 to permit other investigators to elucidate the nature of this material.

After a brief discussion of the human assay method for Krebiozen, data on various animal tumors are presented indicating no effect in mice and equivocal results in 16 dogs and 2 cats—all unconfirmed by other investigators.

Clinical observations are then presented briefly and tabulated in 8 tables. It is impossible from the data furnished and the method of presentation to come to any definite conclusions regarding the efficacy of Krebiozen. A total of 687 patients have been treated, but for analysis these are broken into various smaller groups, i.e. observations on pain, narcotic requirements, psychologic effect, euphoria, objective criteria, et cetera, never totaling to the above 687, admittedly, "because the records are incomplete." Some of the brief clinical abstracts cited to emphasize the usefulness of Krebiozen speak for themselves. The following example will suffice: "Among the 15 patients who had signs and symptoms due to a brain tumor or a post-operative recurrence, or metastasis to the brain, and who were disoriented or in coma, 13 (86 per cent) became lucid after Krebiozen, and remained so for from 48 hours to 21 months (average 3.0 months)."

The statistical data are not broken down into various diagnoses, nor do we know the duration of disease, previous therapy, and its effect. We are told only that all but 7 patients were in the advanced, "hopeless" stage. The duration of positive changes is not stated. Only two tables describe the objective changes. Table 5 is a summary of Dr. Ivy's personal series of 100 patients. Sixty-seven showed "general objective changes." The other factors observed were confined to various *local* aspects and their change, i.e., "induration of edema surrounding the tumor or metastases," 32 of 34 patients (92 per cent), "mobility of an extremity," improved in 27 of 31 patients (87 per cent), "body weight," increased in 37 of 58 patients (63 per cent), "cough," "dyspnea," et cetera. None of these objective changes meets the criteria generally acceptable as evidence of tumor regression. "Change in size of primary tumor or metastases," occurring in 37 of 68 (53 per cent) of the patients would be an acceptable objective criterion if one knew the nature of the cancer, the degree of regression, and the duration of improvement. It is a common ex-

[1] Italics are the reviewer's.

perience for malignant tumors to regress in one area or another for a brief period of time. It is a telling argument against the usefulness of Krebiozen that the most accurate parameter of objective improvement, namely an objective roentgenographic change, was not seen in a single patient of these 100. Table 6 summarizes a portion of the objective data of a series of 587 patients collected by 225 physicians (about 2 patients per observer!). Because the total number of patients observed is not evident and because the same unacceptable objective criteria are used, the data can again not be evaluated properly. Parenthetically, we wondered why each of the 225 reporting physicians confined his efforts with this supposedly useful drug to one, two, or at most a few of his cancer patients.

Much discussion and table 3 are devoted to prove the fact that Krebiozen could have *no* placebo effect. It is assumed naively that, because it was possible to establish "a random Control Group" of patients who did not know what medication they were receiving, these patients' improvement was wholly due to drug effect and not due to psychologic mechanisms. It has been repeatedly pointed out by many observers that *any* measure taken to help cancer patients will improve some of them temporarily, at least subjectively.

The methodology of diagnosis in all of these cases is certainly to be questioned. It is alleged that all 100 cases in Group 1 were biopsy proved. Figure 4 shows the roentgenogram of the stomach of a patient (Case *11*—1) with "probable linitis plastica," diagnosed "by an experienced and well known radiologist" but not by biopsy. A follow-up film is shown to illustrate a normal stomach "of the same patient four months after the start of Krebiozen." The implication is that Krebiozen has produced a remission of four and one-half years' duration in a patient with gastric cancer. It seems to us that a number of the diagnoses in other patients could similarly be questioned.

Of the 687 patients reported, the above patient and 22 others survived four or five years with Krebiozen therapy. Some of these patients, however, had previous or coincident radiotherapy, hormones, or surgery, while re-biopsy or autopsy done in a very few showed persistent carcinoma.

It is of interest that nowhere in their analysis or discussion do the authors make mention of the one hundred unselected cases treated with Krebiozen in seven large tumor clinics (including their own) and reported in the *Journal of the American Medical Association*, October 27, 1951, *147*, 864–873. The committee reported "apparent objective evidence of improvement . . . in only two patients." It was believed that "among the 98 cases with negative response there were several patients . . . in whom temporary or subjective response might have led less experienced observers to report definite objective response."

After reading this 1956 report we would still agree today with the editorial statement concerning Krebiozen published in the *Proceedings of the Institute of Medicine of Chicago* (April 15, 1953, *19*, 282–284) that there has been presented no good evidence of cure or of prolongation of life, or of histologic changes or roentgenographic changes of regression.

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Fluoroscopy in Diagnostic Roentgenology. By Otto Deutschberger, with an Introduction by Frank J. Borelli. Pp. 771, 888 illustrations, W. B. Saunders Company, Philadelphia and London. Price, $22.00.

The author, although ambitious and obviously willing, has bitten off more than could or should be chewed.

This door-stop–sized textbook treats the entire field of radiology through a discussion of but one of its diagnostic media, fluoroscopy. The author, regardless of his stated intent,

has obviously written a textbook of radiology. If fluoroscopy were to be employed routinely in the study of half the clinical conditions covered in this book, there might be good reason to consider the genetic consequences. Chapter 7, the "Hazards of Fluoroscopy," is but eleven pages in length but, unfortunately, contains serious inaccuracies.

The author discusses the use of fluoroscopy in a number of procedures such as the reduction of fractures and the removal of foreign bodies. Until fluoroscopic image amplification has progressed to an extent that such procedures may be conducted with safety to operating personnel, it would probably be wise to refrain from their discussion.

Undoubtedly, the book is the product of sincere and painstaking labor on the author's part. It is, therefore, doubly regrettable that, for the reasons stated above, this reviewer is forced to the conclusion that it should never have been placed upon the market in its present form. It is urged that a second version entitled *Diagnostic Roentgenology* be substituted and that in this version fluoroscopy be placed in its proper perspective. If the biological effects of radiant energy are to be discussed at all, they should be treated more accurately.

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LES BACILLES DE KOCH INCOMPLÈTEMENT ÉVOLUÉS DANS L'INFECTION TUBERCULEUSE. By J. Nègre and J. Bretey. Pp. 88, Masson et Cie, Paris, 1955.

In 1926, Calmette and Valtis had described a benign tuberculous infection in guinea pigs inoculated with cultures and pathologic materials filtered through porcelain candles (Chamberland L_3). These atypical infections were ascribed to prebacillary filterable forms of tubercle bacilli.

On the other hand, Nègre and Bretey observed that guinea pigs inoculated with unfiltered pathologic materials showed a marked allergy, but no demonstrable bacilli by microscopic examination or culture. The only lesion in guinea pigs was a slight enlargement of bronchial and medial lymph nodes. Such results are obtained only by inoculating blood, urine, pleural exudates, foci of osteitis and skin lesions from patients at the beginning of the infection. They also have been observed when studying, by similar methods, the lymph nodes of newborns from tuberculous mothers.

By injecting guinea pigs with very small doses of a young culture of tubercle bacilli (less than eight days old), Nègre and Bretey were able to reproduce the same type of infection in guinea pigs. These young and incompletely developed bacilli were selected from a growing culture by using an ingenious technique.

The bacilli have been shown by Asselineau to have a low lipidic content. This fact explains why they do not incite the formation of nodular lesions, do not multiply on egg media, and are not acid fast.

These abnormal bacilli are never found in sputum or in the pulmonary tissue, presumably because of the high lipid content of such tissue. On the other hand, Nègre and Bretey have shown that the repeated administration of lipids to guinea pigs infected by these incompletely developed bacilli resulted in a typical tuberculous infection.

This new concept of the role of incompletely developed tubercle bacilli could explain some important features of the spread of tuberculous infection and its localization in various organs. The present publication by Nègre and Bretey of their experimental work and the conclusions should stimulate investigation and suggest practical applications, especially in the field of diagnosis in various countries. The original experiments of Nègre

and Bretey have been performed with guinea pigs. It will be of value to attempt to reproduce their results in other host species.

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BOOKS RECEIVED

BLOOD TRANSFUSION IN CLINICAL MEDICINE. Second Edition. By P. L. Mollison. Pp. 587, Charles C Thomas, Springfield, Illinois, 1957. Price, $9.00.

NAPT HANDBOOK. Fifteenth Edition. Edited by Harley Williams and Elizabeth Harrison. Pp. 365, National Association for the Prevention of Tuberculosis and Diseases of the Chest and Heart, London, England, 1957. Price, $9.00.

J. A. M. A. CLINICAL ABSTRACTS OF DIAGNOSIS AND TREATMENT. American Medical Association. Intercontinental Medical Book Corporation with Grune & Stratton, Inc., New York and London, 1957. Price, $5.50.

GUIDE TECHNIQUE ET TOPOGRAPHIQUE D'EXPLORATION BRONCHOLOGIQUE. By L. Duchet-Suchaux and A. Pinelli. Pp. 114, 66 figures, Masson et Cie., Paris, France, 1956. Price, 1,400 fr.

UNSETTLED CHILDREN AND THEIR FAMILIES. By D. H. Stott. Pp. 240, Philosophical Library, New York, 1956. Price, $6.00.

TOPOGRAPHISCHE AUSDEUTUNG DER BRONCHIEN IM RÖNTGENBILD. By Claus Esser. Pp. 210, Georg Thieme Verlag, Stuttgart, 1957. Price, $13.80.

HISTORIA DA LEPRA NO BRASIL. Vol. III. Periodo Republicano (1890–1952). By Heraclides-Cesar de Souza-Araujo. Pp. 715, Departamento de Imprensa National, Rio de Janeiro, Brasil, 1956.

American Trudeau Society

Medical Section of the National Tuberculosis Association

Obituaries

WILLIAM DODGE FROST

1867–1957

William D. Frost, one of the ten honorary members of the American Trudeau Society, passed away in Madison, Wisconsin, on January 25, 1957, at the age of eighty-nine years.

Dr. Frost was not a physician, but his para-medical accomplishments during a period of sixty years would make any physician envious, just as it made the American Trudeau Society proud to honor him. There were several phases in his life, and they have so overlapped that they cannot be considered in exact order.

Dr. Frost was a native of Minnesota, received his B.S. and M.S. degrees from the University of Minnesota, was brought to the University of Wisconsin by Dean Harry Russell, with whom he successfully worked to eradicate bovine tuberculosis. He then began work in bacteriology of the soil, and established a course in communicable diseases. He developed the "cellular test" for pasteurized milk, and the "little plate" method for counting bacteria in milk. He defined the transmission of infections mastitis of cattle to humans.

He was not only a pioneer but a crusader. In 1907 he collected materials for a graphic exhibit on tuberculosis, and the following year was sent with it to Washington for the International Congress on Tuberculosis. It was viewed as a premier collection by Koch, Trudeau, and other workers of that time. It was then enlarged and sent throughout Wisconsin, with lectures later sponsored by the University Extension Service.

Dr. Frost's basic interest was teaching. He taught agricultural bacteriology for forty-three years after 1895, becoming Professor in 1915 and Emeritus Professor in 1937. He had received his doctorate in public health from Harvard in 1913. He helped found the Medical School in 1906 through the Hygiene Committee, which he had helped to organize. He was the author of 90 scientific papers and several textbooks. President Edwin Fred of the University of Wisconsin called Dr. Frost "the last remaining of that hardy band of pioneering scientists" who had raised the school to prominence.

The greatest star in Professor Frost's research crown was his imaginative work in suggesting the mechanism of "antibiosis." Dr. Selman Waksman, who discovered the use of streptomycin and coined the word "antibiotic," said in 1952 that "the development of the field of antibiotics . . . owes a great deal to Dr. Frost's outstanding contribution early in the century, when he laid the basis for our knowledge of the production of specific substances by certain bacteria which inhibit the growth or destroy other bacteria". Sir Howard Florey, discoverer of the use of penicillin, noted in his Lister Memorial Lecture to the Royal College of Surgeons in October, 1945, that "In 1904 Frost published an important paper in which he described as many as seven different technical procedures for the investigation of bacterial antagonism, both on solid and liquid media. One of his most interesting techniques was (the use of) a collodion membrane (to separate the portions of the cultures)."

Dr. Frost was honored in 1954 by the Wisconsin Anti-Tuberculosis Association as a founder of that group in 1908, a president, and a member of the Board for many years.

He was said to represent "The People Against Tuberculosis." He was given a testimonial by the Board of Directors of Morningside Sanatorium, Madison, on his retirement as president in 1952. He had helped to found it forty-three years before, to build it, and directed it since 1933. It has shifted from the care of adults and children so that 95 per cent of its census is children. Dr. Frost helped to organize the Madison Anti-Tuberculosis Association in 1908, before the state association was formed, and used it to make a survey of schools and other sources which disclosed 200 cases and the need for special hospital facilities. He also conducted the first Christmas Seal sale in Madison.

He has most recently been in retirement, with his "ailments and honors." His friends and colleagues of the past twenty years remember him as a short stocky man, wearing a mustache and goatee, with a twinkle and a drawl, and constant and helpful at his philanthropic chores.

Dr. Frost was active in the American Public Health Association, the Society of American Bacteriologists, and the American Association for the Advancement of Science. He is survived by his wife, Minnie Wallace Frost, two sons, Russell and Theodore, a sister Ellen, and numerous grandchildren.

Dr. Frost was not only a quietly dynamic man of his times, but one who was often many years ahead of them and who finally added a whole career of services after his retirement from research and teaching.

W. H. O. Jr.

HARRY A. PATTISON

1827–1957

Harry A. Pattison, an early leader in rehabilitation of the tuberculous, died in Hudson, New York, on February 14, 1957. Older members of the American Trudeau Society (when it was still known as the American Sanatorium Association) recall him as the medical field secretary and supervisor of medical social service of the National Tuberculosis Association. He entered into this phase of his career in 1917, only a few years after obtaining medical degrees from both the Chicago Homeopathic and the University of Illinois medical schools, remaining with the National Tuberculosis Association until 1926. During this period he helped to organize the Cattaraugus County Health Department, serving it as Health Commissioner. In 1926 he supervised construction of the Potts Memorial Institute at Livingston and remained with it until he retired in 1955 to become medical director of the Potts Memorial Foundation.

He developed his great interest in directing philanthropy toward rehabilitation of tuberculous patients at the Potts Memorial Institute. He was also a member of the Medical Council of the Veterans Administration, consultant to the Veterans Administration Hospital at Castle Point, delegate to the International Union Against Tuberculosis at the Hague in 1933, adviser on tuberculosis to the Andrew Mellon Committee on Hospitalization of Veterans, to the Bureau of War Risk Insurance, and to the Federal Board of Vocational Education. As such he worked constantly for adequate rehabilitation measures. He wrote widely on this subject, ultimately publishing his views in a book, *Rehabilitation of the Tuberculous*.

Dr. Pattison's enthusiasm for furthering the work of restoring tuberculous patients to a useful place in society continued until the time of his final illness and death from a cerebral hemorrhage. Present-day workers in this field owe much to his persistence and depth of vision in the earlier decades of this century.

S. J.

NOTICES

The following dates and locations of the next two annual meetings of the National Tuberculosis Association and the American Trudeau Society have been selected and approved:

1958—Philadelphia, Pennsylvania—May 19–24
1959—Chicago, Illinois—May 24–29

ANNUAL MEETING OF THE AMERICAN TRUDEAU SOCIETY

The Medical Sessions Committee INVITES SUBMISSION OF PAPERS on all scientific aspects of tuberculosis and nontuberculous respiratory and cardiopulmonary diseases for presentation at the *1958 annual meeting in Philadelphia, Pennsylvania, May 19–21.*

Abstracts

1. Abstracts of papers to be offered for presentation should be submitted before January 10, 1958, to the Chairman of the Medical Sessions Committee. *Five copies* are required for distribution to committee members.

2. Abstracts should be limited to a 300-word summary including important data *and conclusions*. Charts and tables should be attached to aid in the selection of papers.

3. The names, academic degrees, titles, and complete addresses of the senior author and co-authors should be included in each abstract. The author who is to present the paper should be the first author listed in the abstract.

4. All abstracts will be reviewed by the Medical Sessions Committee. Selection will be largely based on the areas of interest suggested by you and others receiving this invitation.

5. Those wishing to present subjects of a confidential nature from the armed forces or other sources should first clear the material through official channels.

6. Authors will be notified by March 1, 1958, if their papers have been accepted for presentation.

7. Papers presented at the annual meeting are expected to be original contributions not previously presented or published. Medical papers presented at the meeting may be submitted to THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES by the author if he so desires.

Suggestions

The Medical Sessions Committee is particularly anxious to build the 1958 program around your special interests. A memorandum suggesting subjects and speakers would be welcome and should be sent to the Chairman by October 15, 1957.

KATHARINE R. BOUCOT, M.D., *Chairman*
Medical Sessions Committee
Woman's Medical College
Philadelphia 29, Pennsylvania

SCHEDULE OF LABORATORY REFRESHER TRAINING COURSES

July, 1957–June, 1958

The following schedule of laboratory refresher training courses will be offered by the Laboratory Branch of the Communicable Disease Center, Chamblee, Georgia.

Information and application forms should be requested from Laboratory Training Services, Communicable Disease Center, U. S. Public Health Service, P. O. Box 185, Chamblee, Georgia.

Dates	Course Number	Courses	Duration	Location*
			weeks	
Sept. 9–Oct. 4	8.00	Laboratory Methods in the Diagnosis of Parasitic Diseases Part 1. Intestinal Parasites	4	1
Oct. 7–25	8.01	Laboratory Methods in the Diagnosis of Parasitic Diseases Part 2. Blood Parasites	3	1
Oct. 14–25	8.20	Laboratory Methods in the Diagnosis of Viral and Rickettsial Diseases	2	3
Oct. 28–Nov. 1	8.26	Laboratory Methods in the Diagnosis of Rabies	1	3
Jan. 6–24	8.15	Laboratory Methods in Medical Mycology Cutaneous, Subcutaneous and Systemic Fungi	3	1
Jan. 20–31	8.55	Laboratory Methods in the Diagnosis of Tuberculosis	2	2
Feb. 3–14	8.17	Laboratory Methods in the Study of Pulmonary Mycoses	2	1
Feb. 10–21	8.40	Laboratory Methods in the Diagnosis of Bacterial Diseases Part 1. General Bacteriology	2	2
Feb. 24–28	9.40	Laboratory Diagnostic Methods in Veterinary Mycology	1	1
Feb. 24–Mar. 7	8.41	Laboratory Methods in the Diagnosis of Bacterial Diseases Part 2. General Bacteriology	2	2
Mar. 10–21	8.50	Laboratory Methods in the Diagnosis of Bacterial Diseases, Enteric Bacteriology	2	2
Mar. 10–21	9.41	Serologic Methods in the Diagnosis of Parasitic and Mycotic Infections	2	1
Mar. 10–21	8.20	Laboratory Methods in the Diagnosis of Viral and Rickettsial Diseases	2	3
Mar. 24–28	8.26	Laboratory Methods in the Diagnosis of Rabies	1	3
†	8.05	Laboratory Methods in the Diagnosis of Malaria	2	1
†	8.21	Special Training in Virus Techniques	2–4	3
†	8.42	Typing of *Corynebacterium diphtheriae*	1	2
†	8.51	Special Problems in Enteric Bacteriology	2	2
†	8.52	Phage Typing of *Salmonella typhosa*	1	2
†	8.53	Laboratory Methods in the Diagnosis of Leptospirosis	1–4	2
†	8.54	Serologic Differentiation of Streptococci	2	2
†	8.56	Bacteriophage Typing of Staphylococci	2	2

* Location of classrooms: (1) Bldg. 27, Chamblee, Georgia; (2) Bldg. 30, Chamblee Georgia; (3) Virus Laboratory, Montgomery, Alabama.

† Courses given by special arrangement only.

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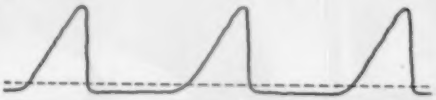

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This book would be a valuable addition to any medical library. It should be on the reference list of every medical school; and on the desk of all private physicians interested in the problems of tuberculosis.

Not only does it supply a historical background for the antituberculosis movement in the United States, but it affords enlightening orientation to the entire voluntary health movement with the National Tuberculosis Association as the principal illustration.

The three overlapping areas of the Association's activities—medical science and practice, public health, and social welfare—are presented here by an able medical historian who knows how to give facts in a clear and interesting way.

Among the chapters are: Medical Background; Social Background; Tuberculosis in the United States; 1904; Founding of the National Tuberculosis Association; Arousing the Public, 1904–1908; Growth of the Tuberculosis Movement, 1904–1920; Administrative Developments and Reorganization; Problems and Policies, 1908–1922; Widening Contacts and Activities, 1917–1922; Foreign Contacts, War, and the Emergence of a New Era; The Research Program, 1940–1954; Chemotherapy and the Climax of Fifty Years of Service.

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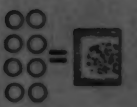

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